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ANNUAL REPORT

DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

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VOLUME II PART 1

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this increase in metabolic rate during the phase of active bingeing and vomiting are currently under study.

Our data also indicate that patients with anorexia nervosa and bulimia nervosa show obsessional symptoms in the range of severity seen in patients with classic obsessive compulsive disorder. These include behaviors such as ritual cleaning, trichotillomania, and repetitive checking. Moreover, their intense preoccupation with food intake and body image can be construed as an obsessional symptom. We have previously reported in *The New England Journal of Medicine* that patients with anorexia nervosa show a profound disruption in the osmoregulation of plasma arginine vasopressin associated with hypersecretion of this peptide into the cerebrospinal fluid. In the light of the fact that centrally-directed vasopressin in experimental animals delays the extinction of behaviors acquired during aversive conditioning, we postulated that the hypersecretion of vasopressin into the CNS could contribute to the obsessive preoccupation that patients with anorexia nervosa have with the potential adverse consequences of eating and weight gain. In the past year, we have shown that normal weight patients with bulimia nervosa show qualitatively similar abnormalities in the osmoregulation of plasma arginine vasopressin and in the secretion of this peptide into the CSF. Recently, we have shown an almost identical defect in patients with classic obsessive-compulsive disorder. These data suggest that the eating and obsessional disorders share similar pathophysiological features and that a common defect in the regulation of arginine vasopressin secretion may underlie the obsessional behaviors that are the cardinal manifestations of these illnesses.

We have recently completed a series of studies exploring neural mechanisms underlying hunger and satiety in patients with the eating disorders. Our data show that patients with both bulimia nervosa and anorexia nervosa show defects in hunger and satiety that either confer susceptibility to their illnesses or complicate recovery. As an example, we showed that patients with bulimia nervosa showed deficient CCK secretion and subjective satiety during the consumption of a normal sized meal that correlated strongly. However, during a binge-sized meal, CCK secretion and subjective satiety were normal. These data suggest that the pathological eating behavior in patients with bulimia nervosa, perhaps as a partial consequence of metabolic and obsessional factors, promoted secondary changes that reinforce abnormal patterns of food intake. The data showing deficient CCK responsiveness during food ingestion in bulimia nervosa is of interest in the light of our pre-clinical data showing that peripherally-administered CCK promotes the acute release of hypothalamic CRH. We postulate that the depressive features of bulimia nervosa, including lethargy and hyperphagia, may reflect hyposecretion of endogenous CRH, either intrinsically or, in part, as a consequence of defective CCK secretion. In addition to our data regarding abnormal CCK secretion in patients with bulimia nervosa, we also have found either primary or secondary changes in the secretion of a variety of neuropeptides that influence hunger and satiety, including NPY, PYY, and beta-endorphin.

One additional approach we have taken to the study of patients with eating disorders has been to compare and contrast pathophysiological mechanisms in these subjects and those with major affective disorders. As an example, in back-to-back original articles in *The New England Journal of Medicine*, we recently showed that the hypercortisolism in patients with anorexia nervosa and melancholic depression had a similar etiology in the hypersecretion of CRH, and that this defect could contribute to many of their common clinical and biochemical manifestations.

Future Directions:

Further study of the neuroendocrinology of obsessionalism: Potential Role of Arginine Vasopressin in the Obsessional and Eating Disorders:

1. Studies of plasma and CSF AVP secretion

Our group first applied techniques to study osmotically-mediated plasma AVP in patients with psychiatric disorders, demonstrated the reproducibility of plasma AVP responses to hypertonic saline in controls, and first reporting the concomitant secretion of dynorphin and AVP during osmotic stimulation in humans. However, previous clinical case reports in patients with disparately located focal hypothalamic neoplasms have revealed either isolated osmotically-mediated defects in the secretion of plasma AVP with intact hemodynamically-mediated AVP secretion, or vice-versa. These data indicate that AVP neurosecretory neurons receive afferent inputs from focal sites that process discrete physiological information. Identification of a possible concordance between CSF AVP secretion and either osmotically or non-osmotically mediated AVP secretion could provide a peripheral marker for centrally-directed vasopressin secretion and will be required for a thorough work-up of AVP secretion.

We are currently in the process of completing a study of the 30-hour pattern of neurohormonal and neurotransmitter secretion into the CSF of patients with bulimia nervosa and would like to extend this study to patients with obsessive-compulsive disorder. These would not only include measurements of AVP per se but also measurements of prepro-AVP, for which we have developed a radioimmunoassay. Recent data suggest that the precursor prohormone levels of neuropeptides in CSF can be much higher than the level of active neurohormones themselves, providing the context for local enzymatic processing of prohormones circulating in the CSF. In addition, one of the enzymes known to be involved in the post-translational processing of preprovasopressin has been cloned and we are in the process of developing methodologies for its quantification. The longitudinal measurement of AVP in CSF is of particular interest because previous data have shown a clear circadian rhythm for AVP secretion into primate CSF.

2. Anti-androgens as a possible means of modifying central AVP and oxytocin function

One of the principal sites of AVP synthesis in the CNS is the bed nucleus of the stria terminalis. It has been shown that estrogen administration or the administration of anti-androgens to experimental animals markedly reduces AVP synthesis in this region. Moreover, estrogen and anti-androgen administration also enhance the synthesis of oxytocin, which has been shown to inhibit the synthesis of PVN CRH. Concurrently, several anecdotal reports have noted that anti-androgens given incidentally to men and women with classic obsessive-compulsive disorder have promoted a dramatic amelioration of obsessional symptomatology. We first propose a trial of anti-androgens in patients with anorexia nervosa. Anorexic patients are invariably amenorrheic, and hence estrogen deficient (i.e. relatively androgenized). This androgenization is exacerbated by their centrally-driven pituitary-adrenal activation, which is associated with an increase in adrenal androgen secretion despite a relative shift of the adrenal cortex away from the androgen to the glucocorticoid pathway.

Parenthetically, Dr. Altemus has recently submitted a report showing that women with obsessive compulsive disorder show dramatic exacerbations in obsessive-compulsive symptomatology either after the cessation of breast feeding, or in the post-partum period, if not breast feeding.

3. The use of a vasopressin antagonist in the treatment of obsessional states

A new non-peptide V2 (central) AVP antagonist (OPC-21268) that crosses the blood brain barrier has been described (Science, 252:572-574, 1991). We have obtained this compound

and have begun to test its safety and biological effects in non-human primates. We have previously obtained IND's for the diagnostic and/or therapeutic use of over a dozen other agents.

4. Studies of the effects of neuropharmacologic agents on site-specific vasopressin mRNA expression in brain

Obsessive-compulsive disorder shows a marked preferential responsiveness to the serotonin uptake inhibitors fluoxetine and clomipramine. In the basic science laboratory, Dr. Altemus is actively studying the effects of neuropharmacologic agents effective in the treatment of OCD on the site-specific expression vasopressin and oxytocin in disparate brain regions. These effects will be compared to other neuropharmacologic agents that are ineffective in the treatment of OCD, including imipramine, phenelzine, and idasozan, as well as other relatively specific serotonin uptake inhibitors such as zimelodine and trazadone. It would be of particular interest if, compared to fluoxetine and clomipramine, the latter two serotonin uptake inhibitors showed systematic differences in their effects on the site-specific expression of somatostatin or AVP mRNA.

Potential Role of Somatostatin in the Obsessional and Eating Disorders:

Like AVP, somatostatin delays the extinction of behaviors acquired during aversive conditioning, is arousal producing, and is hypersecreted into the CSF of patients with obsessive compulsive disorder. Moreover, recent studies show that in contrast to a range of standard antidepressant and anti-anxiety agents, the serotonin uptake inhibitors that are preferential in the treatment of obsessive compulsive disorder cause a significant reduction in somatostatin content in brain, while somatostatin itself has been shown to cause the acute release of serotonin.

1. Studies of the 30-hour pattern of somatostatin secretion into lumbar CSF in obsessional disorders

We hope to study the circadian pattern of somatostatin secretion into CSF in a fashion analogous to our study of AVP in patients with obsessional disorders. This study will be of additional interest in the light of the significant positive correlation in the 9 AM levels of CSF AVP and CSF somatostatin in obsessive compulsive disorder. We are also in the process of immunizing with key sequences from pre-prosomatostatin to explore the secretion of this peptide into lumbar CSF.

Somatostatin has many neuroendocrine functions, including an inhibitory role in the secretion of TSH and GH from the anterior pituitary. Consequently, we plan to concurrently measure the level of these hormones in plasma to explore the possible relationship between their secretion and somatostatin secretion into the CSF, as well as to determine whether possible abnormalities in the circadian organization of somatostatin secretion into the CSF of patients with obsessional disorders is related to abnormalities in either thyroid function or GH secretion.

2. Estimation of somatostatin secretion into hypophyseal portal blood by the continuous infusion of GH-RH in patients with obsessional disorders

Previous studies in volunteers has shown despite the continuous infusion of GH-RH to healthy volunteers and experimental animals, GH is secreted in discrete pulsatile episodes. Pre-clinical data indicate that this pulsatile GH secretion during continuous GH-RH administration is a consequence of pulsatile somatostatin secretion into hypophyseal portal blood. In order to estimate hypophyseal portal somatostatin secretion in patients with obsessional disorders, we propose a study of the effects of a six -hour continuous infusion of GH-RH (1-44) (1ug/kg/hr) on pulsatile GH secretion.

3. Studies of the effects of neuropharmacologic agents on site-specific somatostatin mRNA expression in brain

Dr. Altemus has begun to study of the effects of a variety of serotonin uptake inhibitors on the expression of somatostatin mRNA as assessed by *in situ* hybridization. These drugs not only include fluoxetine and clomipramine, but other serotonin uptake inhibitors as well, in order to ascertain if the expected decrement in somatostatin mRNA and content is general property of all serotonin uptake inhibiting agents or a property specific to clinically efficacious anti-obsessional agents. We shall also explore the effects of our vasopressin antagonist, the antiandrogen flutamide, and a variety of other psychopharmacologic agents on these parameters.

Potential Role of CRH and the pituitary-adrenal axis in the obsessiveness of the eating and obsessive-compulsive disorders

Dr. Altemus has shown that patients with classic obsessive compulsive disorder have increased levels of three CSF peptides (i.e. CRH, AVP, and somatostatin), and that the levels of each of these peptides in CSF correlate positively with one another. Each of these peptides is arousal-producing and could contribute to the symptomatology of a severe anxiety disorder like obsessive-compulsive disorder, and the secretion of each is suppressible by glucocorticoids. The confluence of these abnormalities raises the following several questions that we would like to address on our clinical research unit. These questions, and approaches to their answers, are as follows:

(1) Is obsessive compulsive disorder truly a eucortisolemic state, as suggested by many preliminary studies?

(2) If so, why is the hypersecretion of CRH not associated with hypercortisolism in obsessive compulsive disorder, as well as possible sequelae of hypercortisolism such as the significant reductions in CSF somatostatin and CSF AVP that we have reported in patients with major depression and Cushing's disease.

(3) Does the apparent lack of hypercortisolism in a severe anxiety disorder like obsessive compulsive disorder that is associated with CRH hypersecretion reflect a defect in the capacity of the pituitary-adrenal to generate glucocorticoid mediated negative feedback.

(4) Can the correction of such a putative pituitary-adrenal defect by the administration of exogenous glucocorticoids suppress the hypersecretion of AVP and somatostatin into the CSF in patients with obsessive-compulsive disorder, and if so, is this associated with clinical amelioration?

To evaluate the functional integrity of each of the components of the HPA axis in patients with classic obsessive compulsive disorder, we plan a modified version of the studies conducted in patients with the chronic fatigue syndrome and other putative hypocortisolemic states. These include determination of the 24-hour pattern of plasma ACTH, cortisol (total and free), and vasopressin secretion, assessment of pituitary-adrenal responses to ovine CRH and arginine vasopressin [utilizing the new specific vasopressin analog with relative agonist specificity for the pituitary, d(D-3-Pal)VP], and assessment of the dose-dependent responses of the adrenal cortex to graded doses of ACTH.

To further explore the functional integrity of the pituitary-adrenal axis in patients with obsessive-compulsive disorder in order to determine if there is an abnormality in pituitary-adrenal counter-regulation of the CRH neuron or other components of the stress response, we plan to administer alprazolam for three days prior to the administration of a CRH stimulation test. We have previously shown that alprazolam specifically suppresses the hypothalamic component of the HPA axis without influencing its peripheral elements; by blocking endogenous CRH release and promoting a brief inhibition of pituitary-adrenal function, we should set the stage for a robust pituitary response to exogenous CRH relatively unrestrained by basal cortisol secretion. Such a

paradigm should unmask subtle defects in the capacity of the pituitary-corticotroph to respond to CRH. Assessment of the dose-dependent effects of ACTH on adrenocortical secretion after dexamethasone pre-treatment will allow an estimation of functional adrenal reserve in patients with obsessive compulsive disorder.

If we detect subtle alterations in pituitary-adrenal function in patients with obsessive compulsive disorder, we plan a two week trial of 2X replacement dose glucocorticoids. Such a trial will not cause suppression of endogenous HPA function and should allow the assessment of both behavioral effects and effects of glucocorticoid administration on parameters such as CSF AVP and somatostatin levels.

Assessment of the functional integrity of the HPA axis in other anxiety disorders:

We have previously hypothesized that the abrupt onset and resolution of the profound anxiety associated with panic disorder reflected an activation of the CRH neuron that occurred in the context of basal hypoactivity of the HPA axis; such a context would provide for an acutely disinhibitable CRH system freed of the basal restraining influence of the glucocorticoids; we further postulated that the failure to demonstrate pituitary-adrenal activation during the course of a panic attack was notable in its own right and suggested a possible defect in pituitary-adrenal responsiveness to activation of the CRH neuron. Several lines of indirect evidence were compatible with this formulation, but we considered it no more than a remote possibility until our recent data in patients with obsessive-compulsive disorder showing hypersecretion of CRH into the CSF in the context of apparent eucortisolism. Our data indirectly suggesting a role for the CRH neuron in panic disorder are as follows:

(1) Acute acting agents like alprazolam cause an acute dose-dependent suppression of the CRH neuron without influencing the pituitary-adrenal components of the HPA axis.

(2) Agents whose chronic administration has been shown effective in treating panic disorder such as imipramine and fluoxetine also suppress the CRH neuron after chronic but not acute administration

(3) Graded treadmill exercise (i.e. 50%, 70%, and 90% V02 max) which is a potent stimulus to panic, causes a dose-dependent activation of the pituitary adrenal axis in healthy controls regardless of the amount of work required to reach these levels of maximal oxygen utilization. Pituitary-adrenal activation during exercise correlated closely with plasma lactate levels achieved during the exercise stimulus.

(4) Our in vitro data show that lactate promotes a dose-dependent release of CRH, with the maximal stimulatory dose similar to the concentration of lactate achieved at 90% V02max.

(5) The intravenous infusion of CCK can precipitate panic attacks in susceptible individuals. We have advanced several lines of evidence (vide supra) that the intravenous administration of CCK causes the acute activation of the PVN CRH neuron via activation of CCK receptors located on peripheral vagal afferents.

In the light of these data, we propose a study of HPA function in patients with panic disorder. One of the principal goals of this work is to determine if the failure of the expected pituitary-adrenal activation during the course of a panic attack reflects a peripheral abnormality in either the pituitary or adrenal cortex. We shall apply similar strategies to those outlined in the proposed study of obsessive-compulsive disorder, including stimulation of the pituitary corticotroph cell with CRH after three days of treatment with alprazolam, and evaluation of adrenal reserves with ACTH stimulation after dexamethasone pretreatment. We should also like to evaluate for the possibility of a subtle glucocorticoid resistance by assessing pituitary-adrenal responses to the glucocorticoid antagonist RU 486 and the assessment of local vascular responses to the graded topical administration of dexamethasone. As a caveat, we recognize the possibility of a focal, tissue-specific glucocorticoid resistance and utilize these as preliminary screening procedures. We also propose a study of lumbar CSF to evaluate the secretion of arousal-producing neuropeptides

such as AVP and its prohormone, CRH and its prohormone, and somatostatin and its prohormone. We have developed antisera for the former and are in the process of immunizing with key sequences from the latter two.

Neurohormonal evaluation in obese binge eaters with clinical evidence of obsessiveness:

Dr. Susan Yanovsky of our group has recruited 60 obese women whose body mass index is greater than 30, 130% or more of ideal body weight. Thirty of these women have obesity associated with binge eating disorder, while the remaining thirty do not. Initial work-up has included full psychological evaluation and measurements of body fat distribution [utilizing measures of bioelectric impedance, total body water (deuterium dioxide), and underwater weighing], assessment of metabolic weight, and full psychological evaluation. Initial data indicate that binge eaters score in the range of patients with obsessive-compulsive disorder on the obsessive-compulsive inventory, have a higher percentage body fat, and a lower metabolic rate.

These data raise the possibility that obese binge eaters share pathophysiological features with eating disordered and obsessional patients. To explore possible biological similarities, 15 patients in each group will be studied with lumbar puncture, assessment of plasma AVP secretion, and an abbreviated work-up of the hypothalamic-pituitary-adrenal axis, consisting of CRH stimulation test, ACTH stimulation test, and AVP infusion.

Short-luteal phase defect and infertility in normal weight women: potential effects of dieting and therapeutic implications for a frequent cause of infertility or sub-fertility:

Even subtle chronic food restriction can have effects on neuroendocrine function. Normal weight bulimics who are actively bingeing and vomiting often determine a pattern of food intake and purging that allows them to stay at a normal weight that is nevertheless somewhat below their normal set point; as a possible consequence, they often show oligomenorrhea with a short-luteal phase. We propose that a similar problem may occur in non-bulimic women who present with infertility as a consequence of a short-luteal phase. Accordingly, we hypothesize that a population of infertile women subtle alterations in luteal phase dynamics may show this defect despite the maintenance of a normal weight if their ordinary set-point for weight is somewhat above the normal mean and they are maintaining normal weight by taking in less calories than that dictated by their own set point. In collaboration with Dr. Douglas Rabin, Dr. Altemus is conducting a study in patients with idiopathic short-luteal phase and infertility, diagnosed by both endometrial biopsy and repeated determinations of luteal phase plasma progesterone levels. The first stage of this study is to meticulously evaluate food intake and macronutrient composition, and association with determination of the basal metabolic rate. A group will be selected on the basis of normal weight and a low metabolic weight and placed on a diet consisting of 35% fat and 2,000 calories per day. The impact of this diet on the dynamics of the menstrual cycle and the length of the luteal phase and progesterone secretion will be evaluated.

A second potential approach to this problem is pharmacologic. In the light of data that activation of the CRH neuron can directly (and via POMC derived opiates) produce an inhibition of the LH-RH neuron, we propose a trial of alprazolam in patients with documented short-luteal phase. Such a trial would inhibit the CRH neuron and theoretically disinhibit the LH-RH neuron, whose inhibition could theoretically result in a short-luteal phase defect. We propose that such a pharmacologic intervention would pose fewer potential problems than the prevailing therapeutic intervention for the short-luteal phase, namely the anti-estrogen clomiphene citrate. This agent stimulates gonadotropin secretion by interfering with estrogen's actions in the central nervous system, thereby producing a spurious estrogen-deficient state. It is thought by many that the anti-estrogen effect of clomiphene citrate may itself interfere with fertility.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02509-03 BP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Anticonvulsants in Lithium-Refractory Bipolar Patients

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- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Using the anticonvulsant carbamazepine, we have documented acute response in approximately two-thirds of acutely manic patients and one-third of acutely depressed patients. Lithium augmentation of inadequate response is also observed, in both mania and depression in spite of greater decrements in T_3 and T_4 than with either agent alone. Long-term prophylaxis of both manic and depressive episodes has also been documented with carbamazepine, although a subgroup of patients show loss of efficacy over time. This may represent the development of contingent tolerance; it appears to occur in patients with the most rapidly deteriorating prior course of illness; and it may be reversible with a period of time off medications. Patients who are inadequately responsive to carbamazepine may respond to valproate and vice-versa. Many of the correlates of poor response to lithium (severe, dysphoric mania, rapid cycling, non-familial illness) may be associated with good response to carbamazepine or valproate. Thus, carbamazepine and valproate appear to be important clinical options in lithiumrefractory bipolar illness. We are attempting to elucidate clinical and biological markers of differential response to lithium and the anticonvulsant agents carbamazepine and valproate. Good responses have not been observed to the anticonvulsant phenytoin, while clonazepam may be a useful acute adjunct for breakthrough manic episodes and their associated insomnia. A clinical trial of the anticonvulsant calcium channel blocker nimodipine has been instituted including some patients with ultra-rapid and ultradian cycling mood disorders with promising results in 5 of the first 12 patients treated.

I. Project Description

A. Objectives

Develop new treatment options for patients with lithium-refractory bipolar illness and define clinical and biological markers of response to lithium versus the anticonvulsants as well as among the anticonvulsants, principally carbamazepine and valproate.

B. Methods Employed

Patients are studied in a double-blind fashion and rated by nurses unaware of when or which active compound is being substituted for placebo. Subjects who meet RDC and DSM-III-R criteria for primary major affective illness are admitted to the 3-west Clinical Research Unit of the Section on Psychobiology of the BPB. A variety of clinical and biological studies are garnered during medication-free intervals and then during the course of acute long-term treatment with carbamazepine in order to establish concomitants and predictors of response. For patients with ultra-rapid and ultradian cycling, self and observer ratings are obtained at two-hour intervals.

C. Major Findings

1. Carbamazepine in acute mania. We have documented the acute anti-manic efficacy of carbamazepine in lithium-refractory bipolar patients. There have now been a total of 19 double-blind clinical trials in the world literature, which are consistent with our first double-blind reports of the acute anti-manic efficacy of carbamazepine published in 1978. Preliminary evidence suggests that variables associated with relatively poor response to lithium; i.e., manic severity, dysphoria, rapid cycling and a negative family history of affective disorders in first-degree relatives, may be associated with a good anti-manic response to carbamazepine. We have seen good acute anti-manic response in 13 of the first 21 patients studied (61.9%). Five of six manic patients responded to lithium potentiation, however, at a slower rate than that observed for depression. Preliminary analysis of the data does not support a clear-cut relationship of blood levels of carbamazepine to the degree of anti-manic response. We are continuing to study preliminary observations that the concentration of the active 10,11-epoxide metabolite correlates better with degree of clinical response.

2. Carbamazepine in acute depression

Only approximately 1/3 of patients (17 of 57) studied in a double-blind fashion show a moderate or excellent antidepressant response to carbamazepine in our population of highly treatment-refractory patients. We have now documented that lithium potentiation is highly effective in the inadequate responders to carbamazepine. Eight of 15 acutely depressed patients show rapid onset of improvement following double-blind institution of lithium potentiation. The potentiative effect of lithium occurred in spite of the significantly greater decreases in T_3 and T_4 than observed with either agent alone. During treatment with carbamazepine alone, degree of clinical response was significantly correlated with decrements in T_4 and free T_4 , which is consistent with observations of the group of Baumgartner et al using more traditional antidepressant modalities including chlorimipramine and maprotiline. As studied by R. Herman, we have found carbamazepine-induced decreases in thyroid indices are not accompanied by changes in basal metabolic rate or by alterations in weight, suggesting that the thyroid dec-

rements observed on carbamazepine are not only not typically associated with hypothyroidism (as is observed with lithium) but may actually be markers of better degrees of clinical response.

Even though carbamazepine is an anticonvulsant, to date no evidence exists that this drug is exerting its actions by inhibiting subclinical convulsions in patients with affective disorders. We have observed no evidence of "hot spots" on PET scans and even those with minor EEG abnormalities are not preferentially responsive to the drug. Moreover, those patients with a high incidence of psychosensory symptoms (similar to those observed in patients with complex partial seizures) appear to respond very well to lithium, but these symptoms are not predictive of carbamazepine response. Carbamazepine is the anticonvulsant with the highest "limbic ratio" (i.e., inhibition of afterdischarges emanating from the amygdala relative to those from the cerebral cortex) followed by valproate and phenytoin. In this regard, it is of interest that carbamazepine and valproate appear the most clinically useful in the affective disorders while, to date, we have not observed positive responses to phenytoin in five double-blind trials (albeit in those already preselected for nonresponse to carbamazepine).

3. Carbamazepine Prophylaxis

We continue to observe efficacy of carbamazepine during long-term prophylaxis of both manic and depressive episodes. However, we have observed that a subgroup of patients begin to show reemergence of episodes after one to four years of successful treatment. This reemergence of episodes may represent the phenomenon of tolerance to the psychotropic effects of carbamazepine, similar to that observed in a subgroup of patients with trigeminal neuralgia who are initially highly responsive to antinociceptive effects of carbamazepine. Based on the preclinical data discussed in Z01 MH 02530 BP by Dr. Susan Weiss, we have postulated that the tolerance to carbamazepine may be contingent and not based on pharmacokinetic properties. If this were the case, a period of time off medication might lead to the re-initiation of responsivity similar to that observed in the preclinical model. Preliminary data in several patients studied by Dr. Pazzaglia are supportive of this view and prospective clinical trials of this manipulation are planned. In order to further delineate possible clinical biological markers of response to carbamazepine versus lithium, 53 patients have been entered into a double-blind, randomized, prophylactic crossover treatment with these two agents, followed by a third year of combination treatment. This study, headed by Dr. K. Denicoff, promises to yield important data on whether individual patients are or are not responsive to these two different classes of agents and to possible predictors of this responsivity (see Z01 MH 02514 BP).

4. Valproate: Acute and Prophylactic Efficacy

We now have documented a small series of bipolar patients who respond to one anticonvulsant and not another. In particular, we have observed patients who respond to carbamazepine and not valproate, and vice-versa. Clinical response to carbamazepine or valproate in non-responders to lithium represents a major treatment advance in dealing with refractory bipolar patients.

Using global clinical estimate of response to valproate occasionally used in monotherapy, but often in combination therapy, we have now observed marked acute antimanic responses in 7 of 13 patients, while only 3 of 16 acutely depressed pa-

tients have been this responsive. Similarly, we see a better long-term response in the prevention of manic compared with depressive episodes; 8 of 11 patients have been at least markedly responsive against manic phases, while only 3 of 15 have shown this degree of response against depressive phases. Overall prophylactic responsivity was observed to be complete or marked in six of 12 patients so far followed. In some patients dramatic response has persisted for many years of follow-up. However, preliminary evidence suggests that an occasional patient may also show loss of efficacy with long-term valproate, similar to that observed with lithium and carbamazepine.

5. Carbamazepine-Valproate Combination

Dr. T. Ketter has observed in a double-blind clinical design that a patient who was not responsive to either carbamazepine or valproate when used alone responded to the combination of these two agents. These data, which parallel observations in refractory epilepsy, suggest the two anticonvulsants may exert synergistic effects in affective illness.

6. Lithium

We are currently conducting a study to assess how many of our patients show a tolerance pattern of initial good response to lithium followed by a loss of efficacy thereafter, a phenomenon previously unrecognized in the literature. Our long-term follow-up data focus on the importance of maintenance treatment and an attention to the longitudinal course in an illness that has the potential for malignant progression if left untreated, as well as for the development of tolerance to previously effective treatment agents.

We have also uncovered a novel mechanism related to lithium failure which we have labeled lithium discontinuation-induced refractoriness. That is, in patients well stabilized on lithium, discontinuation may not only lead to the reemergence of new episodes, but also loss of response to lithium once it is reinstituted. This phenomenon could account for the high incidence of lithium nonresponse in those who are noncompliant; i.e., repeated episodes caused by lithium discontinuation could engender refractoriness.

7. Other Anticonvulsants

As noted above, none of the first five patients has shown a good acute or long-term response to phenytoin. We are examining the adjunctive antimanic and antidepressant effects of clonazepam, which appears to be of some utility as a sedating anticonvulsant agent for the treatment of breakthrough episodes and their associated insomnia. However, our recent observations suggest that this drug may be associated with a significant disinhibition syndrome in the occasional patient and further studies are required to elucidate the mechanism of this effect. An acute challenge with the anticonvulsant magnesium (Mg) is planned (in collaboration with D. Rosenstein and T. Ketter) to examine Mg dynamics and possible therapeutic effects. Dr. T. Ketter, in collaboration with the Epilepsy Branch, has been monitoring the mood effects of the investigational anticonvulsant felbamate in patients with partial seizures. Preliminary data suggest that felbamate does not induce psychopathology in this population, and may eventually merit consideration as a study drug in patients with refractory mood disorders.

8. Nimodipine

Although non-dihydropyridine calcium channel blockers dramatically increase carbamazepine levels causing clinical toxicity, the dihydropyridine nifedipine does not have this effect. Our preliminary studies suggest that the dihydropyridine nimodipine also does not have this effect.

We have instituted a double-blind clinical trial of the anticonvulsant and calcium channel blocking agent nimodipine. In 5 of the first 12 patients studied, promising initial responses were observed. In two instances, responses were documented by symptom exacerbation upon placebo substitution and re-response with resumption of active drug in a B-A-B-A design. Some patients with ultra-rapid and ultradian cycling appeared to be most responsive to the drug. We are hopeful that this anticonvulsant with the unique mechanism of action will ultimately provide an alternative or adjunctive treatment option for patients who remain refractory to the existing therapeutic agents and/or are unable to tolerate their side effects.

9. Rational Combination Treatment and Bupropion Kinetics

Dr. T. Ketter has directed studies of carbamazepine and bupropion kinetics and their interaction. He found that carbamazepine decreased bupropion levels while increasing levels of the hydroxybupropion metabolite. In contrast, our preliminary observations suggest that valproate does not have this effect. These observations may explain why, in a few patients, we have observed better clinical responses to valproate and bupropion than with carbamazepine and bupropion.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

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October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of action of the anticonvulsants in the affective disorders

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

While the anticonvulsant and antinociceptive effects of carbamazepine appear acutely, more chronic administration is required for maximum onset of antimanic and antidepressant efficacy. These data suggest that different mechanisms of action may be associated with anticonvulsant, antinociceptive, and psychotropic effects of this drug. Carbamazepine exerts important anticonvulsant actions via peripheral-type benzodiazepine receptors. In addition, unblocked α_2 receptors appear to be required. Antinociceptive but not anticonvulsant effects of carbamazepine have been thought to involve GABA_A mechanisms. Therefore, we undertook a clinical trial of L-baclofen in affectively ill patients and found that it was not only ineffective but may have exacerbated depression. These data suggest the potential utility of exploring GABA_A antagonist strategies in bipolar illness. Dr. S. Weiss has developed an animal model that requires chronic administration in order to demonstrate anticonvulsant efficacy. Thus, the ability of chronic, but not acute or repeated intermittent, carbamazepine to block the development of cocaine- and lidocaine-induced kindling may provide a series of important leads to possible mechanisms of the psychotropic effects of carbamazepine (which also require chronic administration). Based on these studies, we have found that α_2 nor-adrenergic, cholinergic, serotonergic, and somatostatinergic mechanisms are not required and a role for corticotropin releasing hormone has not been definitively demonstrated. A variety of candidate systems remain to be explored including carbamazepine's ability to upregulate adenosine receptors, as well as its effects on other ion channels, neurotransmitter, neuropeptide, and second messenger systems. It is hoped that once the mechanism of action of carbamazepine is elucidated, it might lead to the development of newer, more selective treatment agents for the refractory affective illnesses. Based on a variety of clinical and preclinical findings, we have initiated a study of intrathecal TRH in the treatment of refractory depressed patients.

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I. Project Description

A. Objectives

Examination of the mechanisms of anticonvulsant action of these agents should provide a first approximation to mechanisms of psychotropic action. However, several caveats are required in this regard. The onset of anticonvulsant efficacy to carbamazepine tends to be acute while the onset of antimanic efficacy is often delayed for several days and is not maximum for two or three weeks, and the onset of antidepressant efficacy is often delayed for many weeks and not maximal until a month or more. Thus, the time course of clinical efficacy suggests that anticonvulsant and psychotropic properties of carbamazepine may be dissociable, at least on the basis of temporal parameters, and therefore may be subserved by differential underlying biochemical mechanisms. Nonetheless, the studies aimed at examining the anticonvulsant efficacy of different agents provides a backdrop for studies of putative differences on drug action among the anticonvulsants.

B. Methods Employed

Preclinical studies are conducted in order to examine the acute anticonvulsant efficacy of carbamazepine on amygdala-kindled and other acute seizure models. A novel seizure model that requires chronic carbamazepine administration has been elucidated by Dr. S.R.B. Weiss (see Z01 MH 02528 BP). This paradigm might provide insights into the mechanism of action of carbamazepine, which requires chronic administration in order to demonstrate efficacy; i.e., one that may be more temporally related to the efficacy of carbamazepine in manic-depressive illness. Based on these preclinical models, clinical studies are conducted on patients' biological fluids in order to examine the effects of carbamazepine on putative neurotransmitter, receptor, and second-messenger systems that may be implicated in its psychotropic effects. Finally, based on putative mechanisms of action, novel drug treatments are introduced in order to examine whether a specific mechanism affected by carbamazepine is sufficient to induce psychotropic effects in its own right; i.e., a GABA_A agonist such as baclofen.

C. Major Findings

1. Peripheral-type benzodiazepine receptors

Carbamazepine has been demonstrated to exert anticonvulsant effects not through the classical central type benzodiazepine receptor system (which is the mechanism by which clonazepam and diazepam exert their anticonvulsant effects), but through the so-called peripheral-type benzodiazepine receptor system, which is thought to be linked to calcium channels on mitochondria and, more recently, to cholesterol transport. Evidence that carbamazepine acts on peripheral-type benzodiazepine receptors includes the inability of Ro-15-1788 to block carbamazepine's acute anticonvulsant effects on amygdala-kindled seizures in contrast to the blocking of diazepam's effects. Conversely, the effects of carbamazepine are blocked by the peripheral ligand Ro5-4864, which is ineffective at inhibiting the anticonvulsant effects of diazepam. In addition, the effects of Ro5-4864 are themselves blocked by PK-11195, a selective peripheral-type benzodiazepine antagonist. New evidence provided by Dr. Weiss (see Z01 MH 02530 BP) now further suggests that the peripheral-type benzodiazepine receptor mechanism is involved in the anticonvulsant effects of carbamazepine as cross tolerance occurs between carbamazepine and PK-11195 but not diazepam. Taken together, these data provide the first systematic body of work indicating that the peripheral-type benzo-

diazepine receptor is linked to an important pharmacological effect of a clinically used drug. Prior to this time, the utility of this receptor site had been hotly debated, although considerable work from the laboratory of Dr. E. Costa continues to indicate that this site may be important in the modulation of anxiety and other emotional behaviors, and that an endogenous peptide ligand may exist for this system. We are planning to institute a clinical trial of an agent that works selectively at peripheral-type benzodiazepine receptors in order to see whether its effects parallel those of carbamazepine.

2. GABA_B mechanisms

Work by Terrence and Fromm and colleagues had suggested that the antinociceptive effects of carbamazepine were mediated by GABA_B mechanisms. The evidence supporting this contention included the similarities and structure between carbamazepine and baclofen, the efficacy of l-baclofen in the treatment of trigeminal neuralgia, and the ability of the inactive d-isomer to reverse the antinociceptive effects of both carbamazepine and l-baclofen in the animal model of trigeminal neuralgia. Based on these data, Dr. Weiss examined whether l-baclofen would exert acute anticonvulsant effects on amygdala-kindled seizures and whether d-baclofen would reverse the anticonvulsant effects of carbamazepine. Neither proved to be the case. Thus, we conducted a clinical trial of l-baclofen in depression in order to see whether the psychotropic effects of carbamazepine were more closely in line with the antinociceptive effects of the drug (presumably acting through a GABA_B-like mechanism) or with the anticonvulsant effects of the drug (which do not appear to involve GABA_B mechanisms). Moreover, there was a considerable additional rationale for the examination of l-baclofen in depression as work by a variety of investigators had indicated that chronic but not acute administration of a variety of effective antidepressant modalities up-regulated GABA_B receptors in the frontal cortex of rodents. Recent data from other investigators also suggested that both lithium and carbamazepine upregulated GABA_B receptors in the hippocampus but not the frontal cortex.

L-baclofen was administered to five affectively ill patients in a double-blind clinical trial. None of the first patients showed evidence of clinical response and three of the five patients showed exacerbation of their affective illness during blind treatment with l-baclofen and clinical improvement with placebo substitution. One patient, who showed no response to l-baclofen, subsequently showed excellent acute antidepressant response to carbamazepine, further bolstering the contention that the antidepressant efficacy of carbamazepine is unlikely to be mediated through a GABA_B mechanism.

The initial negative findings in our clinical study not only suggest that the antidepressant actions of carbamazepine are unlikely related to GABA_B mechanisms, but also the likely possibility that prior interpretations of GABA_B alterations in relationship to affective antidepressants may require revision. That is, most investigators have suggested that there may be deficiencies in GABA_B mechanisms and GABA_B agonism was required. In contrast, our preliminary data that patients showed exacerbation of their mood disorder on drug and improvement off drug suggest that relative GABA_B antagonism may be important for antidepressant effects deserves further systematic clinical exploration. This latter interpretation would, in fact, be consistent with the data of Lloyd and associates indicating that effective antidepressant modalities up-regulate GABA_B receptors in frontal

cortex of rats. That is, these agents may cause a relative decrement in GABA_B tone and be associated with compensatory up-regulation of this receptor subtype. Further work remains to be conducted in relationship to both GABA_A and GABA_B mechanisms and their involvement in anxiety and affective disorders. Nonetheless, the current preclinical and clinical studies suggest novel reinterpretations of existing data and provide important preliminary preclinical and clinical evidence suggesting the utility of exploring GABA_B antagonist strategies in the affective disorders.

3. Effects of Carbamazepine on Cholesterol

In collaboration with D. Brown, we have observed highly significant increases in serum cholesterol in patients treated with the drug for two weeks or more. This appears to be related to enzyme induction phenomena, although the precise mechanisms and potential links to the peripheral-type benzodiazepine effects of carbamazepine remain to be explored.

4. Other Putative Mechanisms

A new and important animal model to explore mechanisms of anticonvulsant action with carbamazepine which requires chronic administration (i.e., the use of carbamazepine in cocaine- and lidocaine-kindled seizures) promises to provide important insights into potential mechanisms involved in carbamazepine's psychotropic effects. New data reported by Dr. Weiss suggests that α -2 noradrenergic, cholinergic, serotonergic, and somatostatinergic effects are not critical to this aspect of carbamazepine's action. While CRF potentially reverses the anticonvulsant effects of carbamazepine against cocaine-kindled seizures, this does not appear selective for carbamazepine's effects, as CRF also potentially exacerbates cocaine-kindled seizures on its own. The latter data suggest additional interesting interrelationships of carbamazepine with the hypothalamic-pituitary-adrenal (HPA) axis. Previous data studied in collaboration with Dr. Rubinow had indicated that carbamazepine induces increases in urinary free cortisol in those with normal basal values and escape from dexamethasone suppression. Additional studies in collaboration with Drs. M. Kling and P. Gold have indicated that carbamazepine is capable of blocking local anesthetic release of CRF and has complex effects on the HPA axis in patients, as revealed by CRF tests in patients studied on and off carbamazepine (see Z01 MH 00070-16 BP). Rubinow and associates have demonstrated that carbamazepine significantly decreases CSF somatostatin and the relationship of this action to its spectrum of clinical efficacy in pain, mood, cognitive, and seizure disorders continues to be a promising avenue of exploration. A variety of other candidate systems are being explored in the unique preclinical model (requiring chronic administration of carbamazepine in order to block local anesthetic-kindled seizures) and promising agents will be brought to the clinic for further systematic examination in the near future.

5. Contingent Tolerance

Studies of contingent tolerance to the anticonvulsant effects of carbamazepine indicate that there is cross-tolerance to valproate, suggesting that these two anticonvulsants share a mechanism in common even though effects of valproate peripheral-type benzodiazepine receptors are very weak. Recent data of M. Clark suggest that carbamazepine-induced contingent tolerance may be associated with a decreased up-regulation of GABA_A receptors in the hippocampus, revealing a possible biochemical substitute for the associative tolerance and possibly

explaining the cross-tolerance to valproate which is thought to exert its effects via GABAergic mechanisms.

6. TRH

Evidence indicates that TRH is an anticonvulsant and is hypersecreted (in CSF) during depression. This may be an endogenous compensatory response and increasing TRH (via sleep deprivation or i.v. administration) has been reported to be effective in the treatment of depression in some studies. In order to more directly test this proposition, Dr. L. Marangell has initiated studies of intrathecal TRH (to circumvent the blood-brain-barrier) in refractory depressed patients. The first patient has been treated and experienced no adverse side effects.

7. Nimodipine

Recent data suggest that carbamazepine may exert its actions in part through effects on calcium metabolism. For this and a variety of other rationales, we have studied the therapeutic effects of nimodipine in our affectively ill patients (see #Z01 MH 02509-03). This drug, which is both a calcium channel blocker and an anticonvulsant, shows promise in rapidly cycling, treatment-refractory patients.

8. Anticonvulsant Withdrawal-Emergent Psychopathology and Felbamate

In view of the psychotropic properties of carbamazepine and valproate, the psychiatric effects of discontinuation of these agents are of interest in order to better understand their mechanisms of action and the neurobiology of psychiatric symptoms. Withdrawal-emergent psychopathology has been previously documented with discontinuation of barbiturates and benzodiazepines, but not with discontinuation of phenytoin, carbamazepine, and valproate. In collaboration with the Epilepsy Branch of NINDS we have been monitoring the mood effects of complete discontinuation of phenytoin, carbamazepine, and valproate in patients with partial seizure disorders admitted for telemetry and/or a double-blind trial of the experimental anticonvulsant felbamate. In 21 patients studied thus far, 38% developed moderate to severe symptoms when medication free, predominantly depression and anxiety. Several developed panic attacks and two developed psychosis. In the subsequent felbamate trial, 28% dropped out due to psychopathology, which remitted within two weeks of restarting the original anticonvulsants. Withdrawal-emergent psychopathology was not related to generalized tonic-clonic seizure frequency, but was related at trend levels to simple partial and complex partial seizure frequencies and was more severe in patients with histories of affective/anxiety symptoms or ictal anxiety.

The psychiatric effects of the two-week double-blind trial of felbamate can only be assessed when the blind is broken. However, in patients who went on to chronic open continuation treatment with felbamate, mood ratings generally reflected a lack of psychiatric symptoms in marked contrast to the anticonvulsant withdrawal phase of the study. Thus, the limited data thus far suggests that felbamate may not precipitate psychopathology. We will continue to monitor the psychiatric effects of felbamate to generate pilot data to guide us in the eventual consideration of the use of felbamate in patients with primary mood disorders.

Publications:

Post RM, Ketter TA, Joffe RT, Kramlinger KL. Lack of beneficial effects of l-baclofen in affective disorder, Int Clin Psychopharmacol 1991;6:197-207.

Post RM, Weiss SRB. Sensitization, kindling, and carbamazepine: an update on their implications for the course of affective illness, Pharmacopsychiatry 1992; 25:41-44.

Smith MA, Weiss SRB, Abedin T, Kim H, Post RM, Gold PW. Effects of amygdala kindling and electroconvulsive seizures on the expression of corticotropin releasing hormone in the rat brain, Mol Cell Neurosci 1991;2:103-16.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02511-03 BP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Longitudinal Course of Affective Illness: Implications for Underlying Mechanisms

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PROFESSIONAL:

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CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Methods have been developed to systematically assess and plot the longitudinal course of affective illness in relationship to pharmacological interventions and psychosocial stressors. We are writing a manual for life charting and have computerized various aspects of it. We have characterized a course of illness systematically in this fashion in more than 224 patients with primary affective disorders. These data re-document the early observations that the course is often not only one of recurrences, but of a trend for progressive increases in frequency of cycling and severity of illness in refractory patients. Ultra-rapid and ultra-rapid cycling patterns have also been identified and systematically documented. This cycle acceleration is occurring in the context of a decreasing incidence of psychosocial stressors precipitating affective episodes; i.e., as in sensitization and kindling, they are occurring autonomously. It is against this backdrop that pharmacological intervention must be considered. We have noted that one of the previous traditional interventions for bipolar depression, tricyclic antidepressants, appear to carry a 35% risk of causing definite or likely switches into mania. In those individuals who show this induction, we have observed a pattern of increased rapidity of cycling in the year prior to NIMH admission and longer hospital stays at NIMH, suggesting that TCA-induced switches are a marker if not the cause of a more rapid cycling course of illness. A new phenomenon of lithium discontinuation-induced refractoriness has been described; it is an additional rationale for continuing patients on long-term maintenance treatment. We have begun to assess neurobiological correlates of course of illness using a variety of neurotransmitter and endocrine markers as well as proteins in the CSF. In patients studied after a prolonged period of medication-free evaluation, we have observed that rapid cyclers show significantly higher T_4 and free T_4 levels compared with non-rapid cyclers. These data are contrary to previous notions in medicated patients, where rapid cycling was often associated with hypothyroidism. In bipolar patients, more lethal suicide attempts appear to occur later in the course of illness in contrast with unipolar patients reported in the literature where these often occur after the first episode.

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I. Project Description

A. Objectives

Characterization of the longitudinal course of manic-depressive illness provides unique perspectives for studying underlying mechanisms and the effectiveness of pharmacological interventions. It also highlights the potential malignant nature of the illness with its tendency to show an acceleration in the frequency or severity of episodes as a function of successive episodes. While the tendency for progression has been recognized since the time of Kraepelin, it has received little systematic attention in terms of theory construction or postulates regarding the underlying pathophysiology. For example, the catecholamine and indoleamine hypotheses of affective disorders largely focus on changes occurring during acute episodes. Clearly, given an illness with the potential for recurrences, an acceleration in frequency of cycling, and rapid and continuous cycling, consideration of the long-term course of the illness is critical to its appropriate assessment, investigation, and therapeutic intervention.

B. Methods Employed

We have developed a systematic life-chart method that can be applied rigorously in a retrospective fashion in order to characterize not only minor and major episodes of mania and depression, but the context in which they occur in relationship to important psychosocial events and the accurate assessment of the impact of psychotropic changes. This systematic approach is also delineated in a prospective fashion while patients are hospitalized in the NIMH and followed with both double-blind ratings from nursing observers as well as self-ratings. Longitudinal evaluation of motor activity with activity monitors is also employed. Patients are additionally prospectively followed once they have left the inpatient setting of the NIMH, using a variety of rating instruments, and undergo systematic follow-up by our research social worker G. Leverich. She contacts each patient on a monthly basis in order to update their life-chart methodologies. She, in collaboration with K. Mikalauskas and M. Jones, have developed a computerized methodology for systematic graphic presentation and data management of the life-chart profile. Additional efforts in this regard have been made by K. Denicoff in the outpatient clinic.

With additional funding from the MacArthur Foundation, a manual for teaching the life chart method will be developed with the computerized interface. A chaos-derived equation has been developed by M. Jones and M. George that helps model the course of affective illness.

C. Major Findings

Systematic life charts have been compiled for 224 patients to date. We are using this data set in order to examine a variety of hypotheses regarding the phenomenology, course, patterning, drug responsivity, and prediction of outcome and suicidality in affectively ill patients.

1. Antidepressant-induced switching

For example, we have found that tricyclic antidepressants (TCAs) are associated with definite or likely induction of a manic episode in 35% of our patients, although patients do not show this tricyclic-induced phenomenon on every occasion that they are exposed to TCAs. These data may account for the discrepan-

cies in the literature among reports of definite tricyclic-induced switches and other investigators who do not believe this is a reliable phenomenon. Patients who show definite or likely tricyclic-induced switches were significantly more likely to be rapid cycling in the year prior to NIMH admission, demonstrating 6.9 episodes in that year compared with patients without tricyclic-induced cycles who showed less than four episodes in the year prior to NIMH admission. These tricyclic-induced switchers also stayed at NIMH significantly longer, presumably because they were more treatment-refractory. Thus, the impact of tricyclics in some patients appears to be negative with these agents' at least being markers for a more rapid cycling, potentially refractory, course. What is not clear is whether the tricyclics themselves are implicated in the cycle acceleration or whether they are merely marking patients who are more vulnerable to a more malignant course of illness. In addition, we found that 34% of our patients demonstrated a pattern of acceleration of cycling during treatment with tricyclic antidepressants, findings convergent with those of Wehr and Goodwin who documented this finding in a small numbers of patients. Only 17% of our patients showed a conversion to the continuous form of the illness in relationship to tricyclic administration, as had been postulated by Kukopulos and associates. Generally, similar incidences of switches, cycle acceleration, and conversion to the continuous form, were observed with the monoamine oxidase inhibitors as well.

2. Lithium discontinuation-induced refractoriness

We have uncovered a new phenomenon in the illness. In patients who have been stable on lithium prophylaxis and relapsed upon drug discontinuation, some show a failure to re-respond with reinstitution of lithium treatment. We have labeled this phenomenon "lithium withdrawal-induced refractoriness". It provides an additional reason and rationale for maintenance of long-term lithium prophylaxis, in addition to the findings in the literature and the well-documented clinical observations of investigators such as Angst and colleagues, that up to 80% of well-maintained lithium-treated patients will relapse upon medication withdrawal. Additionally, there is new evidence in the literature that bipolar but not unipolar patients are particularly vulnerable to relapse in the first two weeks of lithium treatment (i.e., a possible withdrawal phenomenon). These data, taken with the new observations of the potential for refractoriness upon reinstitution of treatment, should induce patients and clinicians to be highly conservative regarding the discontinuation of an effective prophylactic treatment in the hope that the illness has "run its course".

In collaboration with K. Mikalauskas and Dr. Pazzaglia, we are exploring the number of patients who demonstrate loss of efficacy to chronic prophylactic treatment with lithium after an initial successful phase of illness, and to what extent repeated lithium withdrawals may be associated with this loss of efficacy as well. Twelve percent of our NIMH lithium-refractory inpatients have shown this pattern of discontinuation-refractoriness. It appears to be associated with a particularly difficult to treat emergence of illness, and some patients, after several years of multiple alternative and adjunctive treatment, have still not recovered. Thirty-one percent of our patients have shown a difficult pattern, that of tolerance development, where initially positive complete or partial response to lithium is followed by gradual loss of efficacy despite maintenance of treatment. As reviewed elsewhere (report Z01 MH 02509), these data on course of illness also provide the backdrop for the consideration of the development of tolerance and

contingent tolerance to the novel anticonvulsant treatment agents that we have helped introduce.

3. Ultra-Rapid and Ultradian Cycling

In collaboration with Drs. K. Kramlinger and M. George, we have identified and followed prospectively a series of patients with ultra-rapid and ultradian cycling. As previously described, rapid cycling (those with four or more episodes in the prior year) occurred in a substantial minority of patients and was associated with relatively poor response to lithium carbonate. However, a series of our patients have cycle frequencies greatly exceeding this magnitude and, in some instances, patients may cycle faster than once every 24 hours. It had previously been assumed that 48-hour cycling (i.e., episodes lasting for one day of depression and one day of mania) was the theoretical limit for cycling in affective disorders. It now appears that cycle frequencies in typical bipolar patients without evidence of personality disorder can clearly exceed this frequency. In many instances, patients had undergone progressive acceleration of their cycle frequency, suggesting that as the illness progresses, reliable and rhythmic frequencies may be lost and faster patterns consistent with random or chaotic fluctuations may develop. Like rapid cycling, these ultra-rapid and ultradian cycling patients may be particularly refractory to treatment and require combinations of lithium and anticonvulsants in many instances.

4. Modeling the Course of Illness with a Chaos-Derived Equation

Mark George and Mark Jones have developed an equation derived from the chaos theory that models the varying life phases of recurrent mood disorders. They analyzed life charts of five recent patients at the NIMH who have progressed sequentially from discrete intermittent illness episodes to ultra-rapid continuous cycling. They developed and used the complex chaotic model, $x_{n+1} = r2(x_n)(1-2|x_n|)$ to analyze cycle frequencies. In this formula, changes in a single variable r delineate distinct illness phases including isolated intermittent episodes ($r=1-2.5$), rhythmic patterns (2.5-4.4), or finally, ultra-rapid chaotic patterns ($r>4.4$). A general pattern of progressive increases in the variable r with some waxing and waning is found in each of these five patients. This model can also generate "theoretical" life charts resembling patients' actual life charts.

This chaos-derived mathematical model, and the definitions of illness phases derived from it, may aid in understanding and classifying the longitudinal course of affective illness. It suggests that alterations in a single biological variable could account for both the progressive increases in cycle frequency and the impact of treatment on course of illness.

5. The Role of Psychosocial Stressors in the Longitudinal Course of Affective Disorders

We have recently completed a review of the literature which strongly supports the view that early episodes are associated with psychosocial precipitants in a high percentage of cases (60%), but with successive recurrences, the proportion of episodes associated with psychosocial stressor decreases (to 36% or less). These statistics were compiled on the basis of 12 systematic studies providing data for this format, although an additional five studies are supportive of this perspective and are consistent with observations by seven other investigators including E. Kraepelin that view the illness as becoming progressively more

autonomous as a function of episode repetition. We have placed these new findings in the context of our preclinical models of sensitization and kindling. In particular, we suggest that as with repeated kindled seizures leading to spontaneous epileptic episodes, the occurrence of repeated precipitated episodes of affective illness may lead to a state where affective episodes begin to occur more autonomously as well. Thus, we are postulating that both stressor sensitization and episode sensitization occur in the course of affective disorders and that both of these phenomena could be mediated by changes at the level of gene expression. As described in detail in the annual report of Rosen, stressors and neurotransmitter systems, as well as seizures themselves, appear capable of inducing the proto-oncogene c-fos. c-Fos appears to be only one of many transcription factors activated by stressors or neurotransmitters and the relative amounts of these substances may subsequently alter neuronal biochemistry and synaptic microstructure in a long-lasting fashion. While it has not been definitively demonstrated as yet, it is thought that the induction of c-fos and other transcription factors secondarily leads to a cascade of events which might involve long-term changes in neurotransmitters, receptors, growth factors, and the structural modifications that may convey increases and decreases in neural excitability based on prior experience.

These data may help conceptualize how psychosocial stressors might be associated with the initiation of longer-lasting neurotransmitter and peptide changes associated with the affective disorders such as evidence for CRH and TRH hypersecretion in depression and decrements in somatostatin as assessed in CSF. Such a perspective also provides a coherent framework for understanding potential mechanisms underlying cycle acceleration, stressor sensitization, and the apparent long-lasting increased vulnerability to further recurrences following initial episodes of affective disorder. Such a conceptualization taken in the context of the positive literature on the efficacy of long-term prophylaxis, additionally suggests the importance of early institution and maintenance of long-term prophylaxis in the affective disorders in order to prevent their potential for malignant progression.

6. Biological characteristics of the course of illness

We are attempting to study the relationship of medication-free biological variables to the course of manic-depressive illness. We are examining CSF and amines and their metabolites, as well as peripheral neuroendocrine data in this regard. We report findings of the relationship of thyroid indices to course of illness as an example of the utility of this particular longitudinal approach. During the patient's medication-free evaluation, we have systematically assessed thyroid function based on levels of T_4 , free T_4 , T_3 and TSH. Samples were collected after an extensive period of medication-free evaluation and, in many instances, some months after the discontinuation of prior lithium treatments. Therefore, these values, in contrast to previous studies in the literature, are highly unlikely to be contaminated by the effect of previous psychotropic medication and goiterigens such as lithium carbonate. We found, contrary to some studies in the literature, that patients with rapid cycling (those with four or more episodes in the year prior to NIMH admission) had significantly higher levels of T_4 and free T_4 during their medication-free evaluations compared with non-rapid cycling patients. Moreover, there was a weak but significant positive correlation with the degree of rapid cycling and the levels of both T_4 and free T_4 .

These data suggest that previous reports of the high incidence of clinical hypothyroidism in rapid cycling patients may have been, in part, an artifact of treatment with lithium and will lead to a reevaluation of the role of thyroid abnormalities in relationship to the course of affective illness. Our data are convergent with the idea that lithium and carbamazepine, which inhibit thyroid dysfunction, are useful in the treatment of this illness. They are also consistent with the high incidence of blunted TSH responses to TRH in affective disorders, presumably reflecting TRH hyperstimulation. In turn, these data are convergent with recent evidence of Nemeroff and colleagues that CSF TRH is actually increased in severely depressed patients. We have initiated a protocol utilizing TRH (administered intrathecally to circumvent the blood-brain-barrier) to assess whether the increased TRH may be an endogenous compensatory mechanism and that additional TRH may have antidepressant effects.

In collaboration with Dr. P. Pazzaglia, we have examined the relationship of CSF protein to acute and longitudinal illness variables. CSF protein is significantly elevated in bipolar I patients compared with controls. This finding is more prominent in males than females. Greater elevations are observed in relationship to a variety of measures suggesting increased severity or chronicity in the course of affective disorders. The mechanistic basis for this effect remains to be further delineated.

As noted above, we will be examining the role of endocrine and CSF amine metabolite variables in relationship to course of illness variables as well as extending prior observations that different phases in the course of illness appear to be associated with differential responsivity to psychotropic agents. That is, considerable evidence indicates that more rapidly cycling patients are less responsive to lithium but may respond to the anticonvulsants such as carbamazepine and valproic acid. Moreover, we have observed that those patients who showed loss of efficacy to carbamazepine after an initial period of good response; i.e., contingent tolerance, showed a more rapid clinical deterioration in the four years prior to institution of treatment compared with those who showed sustained response to carbamazepine. These data provide the backdrop for considering mechanisms that might be related to loss of efficacy of psychotropic agents just as this appears to be a critical issue in cancer chemotherapy, with a very substantial resistance to therapeutic agents that develops to long-term treatments.

Earlier studies in our laboratory have also emphasized the potentially lethal nature of bipolar illness as a function of its longitudinal course. Patients made more severe suicide attempts as a function of duration of illness in contrast to previous reports in unipolar patients where these individuals appear to be at high risk immediately after their initial episodes.

Dr. K. Denicoff has documented the high degree of morbidity associated with bipolar illness even in outpatients treated with lithium or carbamazepine. In his prospective, double-blind, randomized trial, he has observed that patients on either medication are experiencing affective dysregulation approximately one-third of the days each year.

Preclinical data of Dr. S. Weiss suggest that kindled seizures evoke endogenous compensatory mechanisms that are, in themselves, anticonvulsant or enable the anticonvulsant effects of carbamazepine. Thus, biochemical changes may be primary and associated with the pathological process being elicited or secondary and compensatory associated within positive or adaptive changes. Similar distinctions may be critical to unraveling which biochemical changes in affective illness are primary and should be targeted for amelioration, and which are secondary and should be enhanced for better therapeutics of the illness. These data and associated conceptual formulations led to Drs. Post and Weiss being awarded the Ziskind-Somerfeld Prize of the Society of Biological Psychiatry in May, 1992.

Publications:

Altshuler LL, Post RM, Fedio P. Assessment of affective variables in clinical trials. In: Mohr E, Brouwers P, eds. Handbook of clinical trials: the neurobiological approach. Amsterdam, Swets & Zeitlinger, 1991;141-64.

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Post RM, Weiss SRB, Ketter T, Pazzaglia PJ, Denicoff K. Impact of affective illness on gene expression: rationale for long-term prophylaxis, Eur J Neuropsychopharmacol 1991;1:214-16.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

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PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological, Anatomical, and Physiological Correlates of Mood Disorders

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CHECK APPROPRIATE BOXES

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Neuropsychological testing has revealed that affectively ill patients have impaired performance on tests involving implicit memory (priming), the Halstead Category Test, and recognition of facial emotional expression in contrast with unimpaired verbal emotion recognition tasks. The neural substrates of these deficits are systematically being explored by a variety of techniques including testing patients utilizing O_2 blood flow studies with PET methodology. CAT scan studies have revealed increased VBR in patients with affective disorders unrelated to the course of illness, but positively related to age, measures of cortisol hypersecretion, and impairment on the Halstead Category Test. MRI studies suggest decreased area and volume of the temporal lobe in affectively ill subjects compared with controls. Evidence of frontal hypometabolism is revealed on PET scan studies in patients with primary affective disorders as well as depression associated with epilepsy. Temporal and parietal alterations are also evident. Procaine-activation studies reveal marked alterations in psychosensory symptoms with a range of affective changes. Bipolar patients appear particularly prone to euphoric responses, while dysphoric responses occur in relationship to degree of fast activation in the EEG of temporal lobe structures. O_2 PET procedures have been conducted in patients and normal volunteers and reveal procaine-induced increases in blood flow in anterior temporal lobe and/or basal frontal areas as well as in cingulate gyrus. Procaine tests will be continued in patients on and off carbamazepine as well as in cocaine abusers with and without histories of panic attacks.

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I. Project Description

A. Objectives

Intensive study of patients during medication-free and medicated states may provide important information regarding the underlying pathophysiology of the illness and eventually may lead to better treatment interventions.

B. Methods Employed

A variety of neuropsychological tests are employed in order to characterize deficits observed in the illness. Restudy of patients during euthymic intervals in response to medications is also conducted. Anatomical studies are performed using CAT scans and magnetic resonance image (MRI) techniques. PET scans using deoxyglucose and O_{15} measurements of cerebral metabolism and blood flow are also employed. Procaine infusion tests are utilized in order to provide a pharmacological probe of limbic system excitability.

C. Major Findings

1. Neuropsychological Impairments.

Depressed patients appear to demonstrate a variety of deficits on neuropsychological testing. While previous findings in collaboration with Dr. H. Weingartner have demonstrated deficits, particularly on more difficult tasks requiring effortful cognition, recent investigations suggest that deficits are now also observed on "priming" tasks that utilize mechanisms that occur on an unconscious basis. This novel finding, discussed in detail in # Z01 MH _____, has important neurobiological and treatment implications. Moreover, it highlights the notion that depressed patients are not able to overcome their depressive disorders by an act of will, that a deficit exists that in some ways is more severe than that which occurs in other progressive dementias.

Among a variety of neuropsychological tests shown to be abnormal in the depressive disorders, our group has highlighted a series of tests that demonstrate important impairments in visual-spatial function. Patients are markedly impaired on the Halstead Categories Test and this appears particularly prominent in older bipolar patients. These deficits appear to be associated and interact with aging, increases in ventricular brain ratios (VBRs) measured on CAT scans, and indices of hypersecretion of cortisol based on measurements of urinary free cortisol. Consistent with the deficits in the Halstead Categories Test, which depends on abstracting in the visual-spatial modality, patients subjectively report selective deficits in spatial orientation on a systematic cognitive style questionnaire. Other psychological tests that tap temporal lobe and parietal deficits are being further studied in collaboration with P. Fedio and C. Olio. Defects on recognition of emotional facial expressions have also been demonstrated in collaboration with David Rubinow, and new tests of specific aspects of the defect and its relationship to clinical state are being developed in collaboration with M. George. It is noteworthy that depressed patients seem to have particular difficulties with sad and happy affects on these faces and not on other affects which normal volunteers find difficult; i.e., there appears to be some selectivity of affective disorder patients to be unable to appreciate visual affective expression in the modalities in which they are disordered. In contrast to these deficits on facial emotion expression recognition, patients have less difficulty in completing sentences related to verbal recognition of emotions, suggesting, again, the impor-

tance of the visual processing in this illness. A variety of systematic studies are being undertaken to further elucidate the nature of these abnormalities in the neural substrates involved.

2. Neuroactivation PET Studies

In view of the evidence that the limbic structures are the neuroanatomical substrates of emotional experience, we have started a series of neuro-psychological activation studies of patients with affective illness versus normal volunteers targeting the temporal lobes and cingulate gyrus. In these studies, we seek to find, in mood disorder patients, abnormalities in the activation of regional cerebral blood flow (rCBF) that parallel abnormalities observed in neuro-psychological test performance.

We have started to study by oxygen-15 PET scanning techniques the effects on cerebral blood flow of performing facial identity matching versus facial emotional expression matching tasks. Research done by Haxby and colleagues in NIA indicates that facial identity recognition tasks activate the occipital and posterior temporal lobes. We hypothesize that the facial emotional expression matching task will activate the occipital and mid-temporal lobes. Based on our clinical finding of depressed patients having deficits in facial emotional expression recognition, we expect them to display abnormal activation of rCBF during the facial emotional expression matching task.

In the near future we will start studying, by oxygen-15 PET scanning techniques, the effects on cerebral blood flow of performing the Stroop test, in which subjects must state the color in which words are presented while ignoring the distraction of the actual words' being the names of different colors. In normals, this task appears to activate the cingulate gyrus. Patients with affective illness may not perform this task as well as normals. We hypothesize that such patients may also have abnormal patterns of activation of rCBF during this task. We will extend this by also having subjects perform an "emotional" Stroop test, in which they must state the color in which words are presented while ignoring the distraction of the actual words' being the names of unpleasant affects. Again, we hypothesize that patients with affective illness will display abnormal rCBF activation during this task.

3. Anatomy

Systematic assessment of brain size and the ventricular brain ratio has been conducted using both CAT scans and MRI. Initial studies revealed increases in the VBR in depressed patients compared with controls, although this was not related to any of the measures relating to longitudinal course of affective disorders. Using MRI, Drs. L. Altshuler and P. Hauser have found preliminary evidence for decreased volume of the temporal lobe, particularly on the right, and that this correlated with aging in affectively ill patients but not normal volunteers, and with duration of illness as well (even age-corrected). The implications of this finding remain to be further studied both in their own right in relationship to affective disorders, but also as they suggest a possible non-specificity of parallel findings being reported by many investigators in schizophrenic patients.

4. Metabolism

A series of studies of cerebral metabolism and blood flow are being conducted in the BPB. Preliminary evidence has indicated no sign of temporal hyperfunction in a group of patients who appear to respond to the anticonvulsant agents. These data are consistent with the notion that the anticonvulsants such as carbamazepine and valproate are not exerting their therapeutic effects in patients with primary affective disorders by dampening epileptiform discharges in this area of brain. Previous data had demonstrated that spike foci may not show up over the surface EEG when they occur in deep and medial structures of the temporal lobe. We have attempted to systematically explore whether our affectively ill patients show any evidence of a seizure disorder by utilizing EEG studies with sleep deprivation and nasopharyngeal leads. With this methodology we have not found any substantial evidence of EEG abnormalities consistent with preliminary evidence of hypometabolism of the temporal lobes using the PET scan deoxyglucose methodologies. PET findings have also included observations of hypometabolism in the mid-prefrontal cortex and superior posterior parietal areas, similar to that observed in schizophrenics. Temporal regions and left basal ganglia were also lower than in schizophrenics (studied by R. Cohen). Increases in Spielberger anxiety scores correlated with decreases in metabolism in affective patients ($r = .65$, $p < .02$, left medial temporal). Global metabolic rates correlated with severity of Hamilton depression ($r = .63$, $p < .02$).

In contrast to some of the neuropsychological test data implicating right temporal parietal dysfunction in affectively ill patients, studies in seizure disorder patients have suggested the importance of the left temporal lobe dysfunction as well (studied in collaboration with L. Altshuler and W. Theodore). These investigators found that patients with left-sided temporal lobe epilepsy ($n=18$) scored significantly higher on the Beck depression inventory than those with a right-sided focus ($n=21$) or with bilateral temporal foci ($n=20$) or with 16 individuals with generalized absence seizures.

Initial PET data studied in collaboration with E. Bromfield, T. Ketter, and L. Altshuler also suggest that patients with a greater degree of depression associated with their epilepsy had evidence of significant decreases in glucose utilization in inferior-frontal cortex ($p = .0018$) compared with non-depressed patients. While five of the six patients of the original 25 studied who demonstrated significant depression scores on the Beck Depression Inventory had left temporal lobe foci, only inferior-frontal glucose utilization and not temporal metabolism distinguished the depressed from the non-depressed subjects. Thus, a variety of data appear to implicate alterations in frontal glucose utilization in depressed patients whose depression is based on different etiopathological mechanisms (i.e., primary affective disorder or secondary affective disorder associated with epilepsy).

5. Procaine

As an activator of limbic structures, Drs. Ketter and George are utilizing the local anesthetic procaine as a potential probe of limbic system excitability. Preliminary evidence has been garnered in more than 70 subjects including those with primary affective disorders, borderline personality patients (studied in collaboration with Rex Cowdry and Dave Gardner), and normal volunteer controls. Data suggest that procaine is inducing profound psychosensory disturbances

with illusions in essentially every sensory modality. This appears to be dose-related. In collaboration with C. Kellner, M. Kling, and P. Gold, increases in cortisol, ACTH, and prolactin, but not growth hormone, have been observed following procaine infusion. In collaboration with Dr. R. Coppola, topographic EEG studies have been conducted revealing that the patients with the greatest increases in fast activity over temporal areas appear to show the most dysphoric reactions to procaine.

In contrast, patients with bipolar affective disorders appear to show a higher incidence of euphoric responses to procaine than either unipolar patients, borderline patients, or normal volunteer controls. These data suggest a potential unique vulnerability to procaine activation in bipolar patients, potentially implicating alterations in limbic system responsivity. It is also of interest that bipolar patients appear to respond to dopamine-active agents with a higher incidence of euphoria than other subject populations, suggesting the possibility that a variety of pharmacological probes may be able to uncover this vulnerability, now also including local anesthetics.

Recently we have been studying the effects of procaine on regional cerebral blood flow (rCBF) during oxygen-15 PET scans. Seven patients with affective illness, one with cocaine-induced panic disorder, and 16 normal volunteers have been studied thus far. Preliminary inspection of these data suggest that procaine causes a marked decrease in rCBF in the anterior temporal and inferior frontal lobes and the cingulate gyrus, in concert with causing various affective and sensory symptoms. These findings support the proposed role of the limbic system as the neuroanatomical substrate of emotion, and the role of procaine as a limbic activator. Analysis of this data utilizing MRI co-registration and statistical parametric mapping will allow us to explore differences in rCBF responses to procaine across diagnostic groups (in affectively ill patients and cocaine-induced panic disorder patients versus normal volunteers) and across emotional responses (in subjects with euphoric versus anxiety responses). Arterial blood sampling during the procaine-PET scans has allowed us to evaluate the blood gas and pH responses to procaine. Significant decreases in blood pCO₂ and increases in pH have been observed in response to procaine. Blood pH (but not pCO₂) has shown trends toward positive correlations with anxiety and fear, and a negative correlation with calmness, utilizing both absolute and change from baseline measures.

II. Proposed Course of Project

As chronic treatment with carbamazepine has been demonstrated by S.R.B. Weiss (see # Z01 MH 02528-01 BP) to inhibit the development of local anesthetic kindling, we have begun to examine the effects of chronic carbamazepine treatment on procaine response in our affectively ill patients. We also wish to examine the possibility that alterations in procaine responsivity may predict subsequent response to carbamazepine, as has already been reported with acute local anesthetic challenges with lidocaine predicting long-term therapeutic response to carbamazepine in the syndrome of myoclonic tinnitus.

We will also be using the procaine activation test to assess limbic activation in patients with cocaine abuse disorders with and without panic attacks, as studied by T. Ketter. As reviewed elsewhere, preclinical data suggest that the

local anesthetics lidocaine and procaine, which are not psychomotor stimulants because they lack major effects on catechol- and indoleamine neurotransmitters which are activated by cocaine, exert relatively selective effects on limbic system structures. Previous electrophysiological and metabolic studies have indicated that lidocaine seizures are relatively selective for limbic system structures and new data using in situ hybridization of mRNA for the proto-oncogene *c-fos* confirm these findings (studied in collaboration with T. Nakajima, M. Clark, and J. Rosen).

Thus, we will be utilizing the relatively pure local anesthetic procaine in order to examine whether any of cocaine's panicogenic effects seem to be inducing long-term alterations in limbic system excitability. There is much preclinical and clinical data to support the finding that cocaine induces panic attacks through a kindling-like progressive mechanism. T. Uhde and associates have observed patients who do not initially demonstrate cocaine-related panic attacks, but after many repetitions, develop spontaneous panic attacks. This same progression occurs with electrical and pharmacological kindling and may provide important insights into the evolution of this important cocaine-related psychiatric syndrome. We have been combining topographic EEG techniques with studies of regional cerebral blood flow using O_{15} PET in order to further ascertain the neural substrates involved in cocaine-induced euphoria, dysphoria, and panic disorder, and compare these findings with findings from patients with endogenous panic disorder and endogenous affective illness.

Publications:

Altshuler LL, Conrad A, Hauser P, Li X, Guze BH, Denicoff K, Toutellotte W, Post RM. Reduction of temporal lobe volume in bipolar disorder: a preliminary report of magnetic resonance imaging [Letter to the Editor], Arch Gen Psychiatry 1991;48:482-3.

Bromfield EB, Altshuler L, Leiderman DB, Balish M, Ketter TA, Devinsky O, Post RM, Theodore WH. Cerebral metabolism and depression in patients with complex partial seizures, Arch Neurol, 1992;49:617-23.

Kling MA, Roy A, Doran AR, Calabrese JR, Rubinow DR, Whitfield HJ Jr, May C, Post RM, Chrousos GP, Gold PW. Cerebrospinal fluid immunoreactive corticotropin-releasing hormone and adrenocorticotropin secretion in Cushing's disease and major depression: potential clinical implications, J Clin Endocrinol Metab 1991;72:260-71.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02513-03 BP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Therapeutic and Mechanistic Effects of Sleep Deprivation in Depression

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Robert M. Post, M.D., Chief, BPB, NIMH

L. Altshuler, M.D., Mental Health Clinic, Brentwood VA Hospital

T. Ketter, M.D., BPB, NIMH

P. Pazzaglia, M.D., BPB, NIMH

D. Brown, M.D., BPB, NIMH

L. Marangell, M.D., BPB, NIMH

K. Denicoff, M.D., BPB, NIMH

M. George, M.D., BPB, NIMH

COOPERATING UNITS (if any)

Brentwood VA Hospital, Brentwood, CA

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychobiology

INSTITUTE AND LOCATION

NIMH, Bethesda, MD

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOXES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

One night's total sleep deprivation can induce profound antidepressant effects in depressed patients. Following the absence of sleep for one night, 40-60% of depressed patients typically show the transient onset of mood improvement which is then lost following a nap or the next night's recovery sleep. Thus, the sleep deprivation paradigm becomes a non-pharmacological means of acutely inducing altered mood states in a fashion that may yield important information about underlying mechanisms. While the mood changes are usually transient they may, nonetheless, give insights into neural systems involved in mood dysregulation and its acute reversal. Some patients show stable degrees of improvement following sleep deprivations, while others show tolerance and yet others show a relative refractoriness to sleep deprivation-induced mood improvement early in their bipolar depressive episode, but vulnerability to transient or long-lasting switches out of depression later in the course of their episode. These data provide some of the first systematic observations regarding differential neurobiological responsiveness as a function of the course of a bipolar depressive episode. Preliminary data suggest that increases in TSH secretion may be associated with the degree of clinical response to sleep deprivation in our patient population as a whole, as well as individual case studies. Patients with greater degrees of diurnal variation in mood on the baseline day prior to sleep deprivation show greater antidepressant response compared with those without diurnal variation.

Other professional personnel (cont.)

T.W. Uhde, M.D., Chief, Section on Anxiety and Affective Disorders, BPB, NIMH
C. Davis, M.S., Chemist, BPB, NIMH
D. Rubinow, M.D., Chief, Section on Behavioral Endocrinology, BPB, NIMH
T. Brown, M.D., Guest Researcher, BPB, NIMH
D.S. Gill, BPB, NIMH

I. Project Description

A. Objectives

To explore the mechanism involved in the acute antidepressant effects of sleep deprivation in order to gain an insight into the onset and offset mechanisms involved in these rapid mood inductions. Systematic investigation of sleep deprivation may also provide an adjunctive therapeutic tool in the treatment of depressed patients, particularly if given with concomitant antidepressant treatment such as lithium or other pharmacological modalities.

B. Methods Employed

Patients are asked to remain awake for either the entire night of sleep deprivation or for the second-half of the night (4 AM to 8 AM) for partial sleep deprivation in an effort to precipitate acute positive changes in mood that appear associated with this manipulation. Patients are rated systematically by nurses as well as continuing to apply repeated self-ratings in order to assess the time course and magnitude of these mood changes and their reversal during naps and following recovery of sleep.

C. Major Findings

We and others have consistently observed significant acute antidepressant effects following one night's sleep deprivation in some 40-60% of acutely depressed patients. The effects tend to be transient with relapse during a nap or following the first night's recovery sleep. Occasionally, more sustained response and even termination of the depressive episode has been observed. While placebo controls are difficult, observations by others that sleep deprivation in the first four hours of the night is less effective than the second four hours provides some degree of control over the manipulation. Recent data by Van den Hoofdakker suggested that patients with more profound diurnal variation showed better antidepressant response to sleep deprivation. Our findings in 31 depressed patients reveal that those with the greatest degrees of diurnal variation in mood on the baseline day (more depressed in the A.M. than P.M. by two points on the nurses' global consensus ratings) show more profound mood improvement following sleep deprivation.

A variety of data suggest that increases in TSH normally observed following sleep deprivation may be related to the degree of clinical antidepressant response although not all studies agree. Our preliminary data in collaboration with L. Altshuler, T. Ketter, and D. Shapiro suggest that positive sleep deprivation response tends to be associated with greater increases in TSH. Moreover, we are exploring whether changes in plasma cortisol are correlated with response in individual case studies. We have also uncovered the novel finding in bipolar patients that there appears to be a relative refractory period to sleep deprivation response in some rapid cycling patients. In the initial days of a depressive episode, some bipolar patients appear refractory to the sleep deprivation manipulation. However, with continued duration of their depressive episode, they increasingly appear vulnerable to transient sleep deprivation-induced mood improvement or even the precipitation of a switch out of depression and into a euthymic or hypomanic episode. These data provide some of the first systematic evidence for changing biological and environmental responsivity as a function of the duration of a depressive episode. These observations have been submitted for publication.

A variety of neurobiological mechanisms has been systematically explored in earlier studies of this phenomenon (see report of Roy-Byrne et al, 1984). Not only will we now be focusing on potential relationships of endocrine measures and degree of clinical response, but also hope to use O_{15} blood flow studies in order to document potential changes of glucose utilization. These findings become all the more intriguing in relationship to the original observations of Wu, Bunney, and Buchsbaum and associates at U.C. Irvine indicating that those patients who respond to sleep deprivation have increased glucose utilization in limbic structures, particularly cingulate gyrus, which returns to normal following sleep deprivation-induced mood improvement.

Publications:

Gill, DS, Ketter, TK, Post RM. Increasing antidepressant response to sleep deprivation as a function of time into depressive episode in bipolar patients. Biol Psychiatry, in review.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02514-03 BP

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Carbamazepine and Lithium Treatment of Bipolar Illness

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Kirk D. Denicoff, M.D., BPB, NIMH
Robert M. Post, M.D., BPB, NIMH
Terence Ketter, M.D., BPB, NIMH
Earlian Smith-Jackson, R.N., MHANA, NIH
Kimberly Blake, B.A., BPB, NIMH

COOPERATING UNITS (if any)

CP, CC, MHANA, NIH

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Section on Psychobiology

INSTITUTE AND LOCATION
NIMH, Bethesda, Maryland

TOTAL STAFF YEARS: 2	PROFESSIONAL: 1	OTHER: 1
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CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are comparing the prophylactic therapeutic effects of lithium and carbamazepine in a double-blind, randomized, crossover design, followed by a period of treatment with both drugs in combination in outpatients with manic-depressive illness. Each drug phase lasts up to one year. We intend to explore the possible differential clinical efficacy of lithium, carbamazepine, and the combination, and assess possible clinical and biological correlates of response. We have added a fourth (one year) phase to the study consisting of sodium valproate in combination with lithium. We now have entered 53 patients (50 of whom have entered the randomized drug trial) and plan to recruit a total of 60 patients. Patients will have the following tests: hematological and thyroid indices, 24-hour urine collection for urinary free cortisol and neurotransmitter metabolites, TRH stimulation, DST, lumbar puncture, EEG, MRI, EKG, and an extensive battery of neuropsychological tests including assessment of psychosensory symptoms. Patients will also have a detailed life chart of their course of illness. A number of self- and observer-ratings are performed. Thirty-six patients have completed the first drug phase and 26 patients have also completed the second drug phase. In the first phase with lithium, 11 of 17 patients were able to complete the year and 6 of 11 patients failed; one patient had to stop because of severe acne. With carbamazepine, 8 of 19 patients were able to complete the year and 11 of 19 patients failed; 5 patients had to stop because they developed a rash. A total of 37 patients have been administered carbamazepine (this includes patients who have not completed a full one-year phase) and 8 (22%) have had to stop because of a drug rash. Of the 53 patients who have entered the study, 12 have dropped out. Eight patients have entered the fourth arm of the study, consisting of valproate in combination with lithium; several of these are responding extremely well.

Other Professional Personnel:

Gabriele Leverich, M.S.W., BPB, NIMH

McDonald Horne, M.D., Clinical Pathology Department, CC, NIH

I. Project Description

A. Objectives

We are studying and comparing the prophylactic therapeutic effects of lithium with carbamazepine in a double-blind, randomized, crossover design, followed by a period of treatment with both drugs in combination, in outpatients with manic-depressive illness. We intend to further explore the possible differential clinical efficacy of lithium, carbamazepine, and the combination, and assess possible clinical and biological correlates of response. We have a fourth option consisting of a one year trial of sodium valproate in combination with lithium which will allow us to compare the combination of sodium valproate and lithium with the combination of lithium and carbamazepine (as well as the previous monotherapy trials).

B. Methods Employed

Patient subjects are recruited through the NIMH outpatient clinic. All subjects must satisfy the DSM-III diagnostic criteria for manic-depressive illness (bipolar). Patients must have had at least two episodes in the two years preceding the trial and at least one past episode of mania. We are actively recruiting lithium responders to add power to the crossover. Patients will have an extensive history taken with a number of areas covered, such as personal psychiatric history, neurological history, family history of psychiatric and epileptic illness, and medical history. A detailed life chart will be constructed for each patient so that the prior course of illness can be assessed from many perspectives. Data will also be collected on the occurrence of psychosensory symptoms (those often experienced by patients with seizure disorders) and on suicide ideation and attempts using previously validated semi-structured interviews. A physical exam will be done. All subjects will have the following tests: blood count, ESR, thyroid functions, 24-hour urine collection for urinary free cortisol and creatinine, EEG, MRI, and EKG. A number of self and observer ratings will be performed. Patients will fill out self-ratings such as the Daily Affect Scale, the Spielberger Anxiety Scale, and the Beck Depression Inventory. Observer ratings will also be completed such as the Bunney-Hamburg global measures for depression, mania, anxiety, psychosis, the Hamilton Depression Scale, the Brief Psychiatric Rating Scale (BPRS), and the Clinical Global Index (CGI). The life chart process will continue to be completed on a prospective basis.

For patients studied during the medication-free evaluation at NIMH, a series of endocrine, physiologic, and biochemical tests will be conducted. These may include: urinary free cortisol, dexamethasone suppression tests, TRH stimulation test, averaged evoked potentials with topographic mapping, procaine infusion test, and lumbar puncture.

During treatment, patients will have the following tests: drug levels, complete blood counts, and SMAC. Hematologic status will be monitored frequently from the baseline period throughout the study period. The SMAC will be administered every month.

We also plan to retest during each single treatment phase in those patients who consent. The various challenges to be administered will include repeat of dexamethasone suppression test (carbamazepine induces escape from dexamethasone

suppression), TRH stimulation test (carbamazepine blunts TSH response) and procaine infusion. Urine will be tested for items such as: 24-hour cortisol (carbamazepine increases urinary free cortisol) and creatinine. Lumbar punctures will be administered to measure CSF for parameters such as the 5HT metabolite 5-HIAA, the dopamine metabolite HVA, and peptides such as somatostatin (as carbamazepine has been postulated to affect each of these neurotransmitter systems). Electro-physiologic tests will be administered such as EEG, AER and topographic mapping.

The treatment trial will be a partial double-blind crossover in the following order: randomized to start on lithium or carbamazepine then changed to the drug not taken, then the third arm consisting of the combination of lithium and carbamazepine. Patients who fail the treatment trial because of poor clinical response or because of treatment-limiting side effects from carbamazepine, lithium, or the combination, will be offered a fourth option consisting of a one year trial of sodium valproate in combination with lithium. Those patients who are unable to take lithium will be administered sodium valproate only. Patients will be on the first arm of the treatment for one year unless they demonstrate a failure to be stabilized or they relapse. They will then go on to the second arm of the treatment for one year or until they relapse. They will then go on the third arm of the treatment with the same conditions.

C. Major Findings

We now have entered 53 patients (50 of whom have entered the randomized drug trial) and hope to recruit a total of 60 patients by the end of this year. At this time, 36 patients have completed the first drug phase and 26 patients have also completed the second drug phase. The breakdown of the 36 patients who have completed the first phase: with lithium 11 of 17 (65%) patients were able to complete the year, 6 of 17 (35%) failed; i.e., could not complete the phase, and one patient (6%) had to stop because of severe acne; with carbamazepine 8 of 19 (42%) were able to complete the year, 11 of 19 (58%) patients failed, and 5 (26%) patients had to stop because they developed a rash. A total of 30 patients have completed a lithium phase (17 completed 12 months; 13 failed; 2 had to stop because of side effects) and 31 patients have completed a carbamazepine phase (11 completed 12 months; 20 failed; 9 had to stop because of side effects). A total of 37 patients have been administered carbamazepine (this includes patients who have not completed a full one-year phase) and 8 (22%) have had to stop because of a drug rash. Only two patients on either drug have completed a full one-year phase without breakthrough symptoms. In addition, there has been a high need for adjuvant medications (i.e., neuroleptics, antidepressants).

We are analyzing the morbidity of illness in a population of bipolar patients who are in a double-blind, randomized, crossover design comparing the prophylactic therapeutic effects of lithium, carbamazepine and both drugs in combination. Patients are evaluated at least every four weeks utilizing prospective life charting methodology. In this methodology, patients are assessed at each visit for each day since their previous outpatient visit in terms of euthymia, mania, or depression. In addition three severities of affective episodes are categorized based on a functional incapacity criteria (mild, moderate, or severe). In the analysis of the first 360 days of treatment for 33 patients, 32 of 33 patients had at least one day of depression and 30 of 33 patients had at least one day of mania. None of the patients were euthymic for the whole year. The average patient was eu-

thymic for 226 days, manic for 35 days and depressed for 18 days. When this data is analyzed in terms of functional impairment (mean number of days out of the first 360 days): mild depression 65.5, moderate depression 29.7, severe depression 2.5, mild mania 28.6, moderate mania 6.4, and severe mania 0.9.

We have noted that a larger than expected number of patients have B_{12} levels below 300 pg/ml and have started a collaboration with Dr. McDonald Horne (hematology) to investigate this more systematically.

II. Significance to Biomedical Research and Program of the Institute

Several factors have led to the choice of this partial double-blind, crossover study. A critical question to be addressed is whether individuals respond relatively selectively to one drug but not the other or whether responsive patients will overlap considerably. The design of this study allows us to compare lithium and carbamazepine in all the patients. In addition, we will be able to study the possible benefit of the combination of lithium and carbamazepine. As each patient will be used as his own control with similar assessment techniques in each crossover phase, the relative efficacy of each intervention for a given patient will be discernable and allow for subsequent analysis of relationship of response to clinical and biological pretreatment variables and correlates.

III. Proposed Course of Project

We plan to continue to recruit patients until 60 subjects have been entered into the study.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02515-03 BP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clonidine and GRF studies in Pathological Anxiety and Normal Controls

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T.W. Uhde, M.D., Chief, Section on Anxiety and Affective Disorders, BPB, NIMH

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M.E. Tancer, M.D., Department of Psychiatry, University of North Carolina

K. Gadde, M.D., BPB, NIMH

D. Roscow, Guest Worker, BPB, NIMH

M. Geraci, MSW, Dept of Mental Health Nursing Service, CC

B. Scupi, MSW, BPB, NIMH

COOPERATING UNITS (if any)

University of North Carolina; Mental Health Nursing Service, CC

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Anxiety and Affective Disorders

INSTITUTE AND LOCATION

NIMH, Bethesda, MD

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Growth hormone (GH) responses to agents that perturb the hypothalamic growth hormone axis have been widely used in psychiatric research as a peripheral correlate of central noradrenergic activity. "Blunted" GH-responses to clonidine are generally attributed to post-synaptic noradrenergic receptor down-regulation. In addition to clonidine, we have administered growth hormone-releasing factor (GRF), caffeine, glucose, yohimbine, and TRH to panic disorder patients and normal control subjects. Overall, patients with panic disorder appear to have decreased GH function using a wide array of stimuli that activate the hypothalamic-GH axis. Future studies will investigate the GH response to sleep, exercise, pentagastrin, CI-988, and pyridostigmine.

I. Project Description

A. Objectives

To differentiate GH abnormalities among panic disorder patients, social phobics, and normal volunteers.

B. Methods Employed

Patients range in age from 18-60 and meet DSM-III-R Diagnostic Criteria for social phobia or panic disorder. Normal controls are screened for personal history of Axis I psychopathology. Patients are physically healthy as determined by history, physical examination, and clinical pathology results (CBC, SMA-20, thyroid function tests).

Following an overnight fast, subjects come to the clinic at 9 a.m. on two or three occasions. Subjects are maintained on a low monoamine, low caffeine diet for at least 24-72 hours prior to the study. All premenopausal women are evaluated in the follicular phase of the menstrual cycle. In the clinic, an intravenous catheter is inserted in an antecubital vein. At least 45 minutes after IV insertion, subjects receive one of a series of agents designed to stimulate GH release. The GH response to each of these agents is evaluated, in independent studies, under double-blind, placebo-controlled conditions.

The GH response to the following agents represents the current focus of attention: alprazolam, caffeine, clonidine, glucose, GRF, pyridostigmine, yohimbine, pentagastrin, CI988, and CCK-4. The GH response during sleep and after exercise are also being investigated by the SAAD.

Blood samples are obtained at baseline and at selected intervals thereafter, depending on the particular stimuli under study. Most studies employ a post-drug schedule of +15, +30, +45, and +60 minutes following the oral or intravenous administration of drug or neuropeptide.

C. Major Findings

In two separate studies, we have now demonstrated that panic disorder patients have a blunted GH-response to clonidine compared to the normal controls. These observations have been confirmed by three of four independent research laboratories.

Compared with normal controls, the SAAD has also found that patients with panic disorder have blunted GH-responses to GRF, caffeine, and yohimbine. A similar blunted response in the delayed rise in GH secretion has been observed by our Section after glucose administration. We have not observed a paradoxical increase in GH after TRH challenge, as has been previously reported in patients with major depression.

II. Significance to Biomedical Research and Program of the Institute

The blunted GH response to clonidine is consistent with a pre-existing state of noradrenergic overactivity (e.g. leading to secondary α -2 adrenergic downregulation of post-synaptic receptors located in the hypothalamus). Other potential abnormalities in serotonergic, dopaminergic or selective neuropeptide-neuromodulatory systems may account for our findings. It is also possible that there is an intrinsic abnormality in the hypothalamic-GH-somatomedin axis. The theoretical possibility that children with anxiety disorders might have decreased hypothalamic-GH function leading to disturbances in stature or growth acceleration represents a focus of future research. Moreover, the impact of psychotropic agents on hypothalamic-GH function will be investigated. The information derived from these studies is expected to improve our understanding of the neural substrates underlying the important biological functions of arousal, fear, and anxiety. This research effort may also improve our understanding of the role of these neurotransmitter-neuromodulatory systems on physical growth and development.

III. Proposed Course of Project:

Future studies will investigate the mechanisms of decreased growth hormone function in children and adults with panic disorder.

Publications:

Uhde TW, Tancer ME, Rubinow DR, Roscow D, Boulenger J-P, Vittone B, Gurguis G, Geraci M, Black B, Post RM. Evidence for hypothalamo-growth hormone dysfunction in panic disorder: profile of growth hormone (GH) responses to clonidine, yohimbine, caffeine, glucose, GRF and TRH in panic disorder patients versus healthy volunteers. *Neuropsychopharmacology* 1992;6:101-18.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02516-03 BP

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychobiological Correlates and Treatment of Social Phobia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T.W. Uhde, M.D., Chief, Section on Anxiety and Affective Disorders, BPB, NIMH
M.E. Tancer, M.D., Department of Psychiatry, University of North Carolina
B. Black, M.D., Medical Staff Fellow, BPB, NIMH
K. Gadde, M.D., Medical Staff Fellow, BPB, NIMH
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D. Roscow, Guest Researcher, BPB, NIMH

COOPERATING UNITS (if any)

University of North Carolina; Mental Health Nursing Service, CC

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Anxiety and Affective Disorders

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Patients meeting DSM-III-R criteria for social phobia are evaluated using psychological, physiological, biochemical and pharmacotherapeutic methodologies. The SAAD investigates the phenomenology, neuroendocrinology, and treatment of social phobia. Current evidence indicates that social phobia can be distinguished from panic disorder on a number of previously validated rating scales. A discriminate function analysis has yielded an algorithm which can correctly diagnose subjects 80% of the time. These syndromes differ in life-time rates of major depression as well as age of onset and sex-distribution. Preliminary evidence suggests that social phobia may be associated with minor disturbances in noradrenergic and/or autonomic dysfunction, although the clinical significance, if any, of these findings have yet to be determined. The Section found that social phobic patients respond to treatment with phenelzine and alprazolam, although phenelzine is significantly superior to alprazolam on a global measure of work and social function. Preliminary evidence suggests that fluoxetine is effective in the treatment of social phobia. A pilot study has been initiated to test the relative efficacy of imipramine versus phenelzine in the treatment of social phobia.

I. Project Description

A. Objectives

To further characterize the phenomenology, co-morbidity and neurobiology of social phobia. To study and develop new pharmacologic treatments for social phobia.

Phenomenology/Course of Illness Social Phobia

B. Methods

The data for the phenomenology of social phobia research comes from detailed structured psychiatry admission interviews and self-rating data obtained at admission. Patients range in age from 18-60 and meet DSM-III-R Diagnostic Criteria for social phobia. Upon application and at regular intervals after acceptance into our clinical research program, a variety of self-rating questionnaires are completed. These include the Fear of Negative Evaluation Scale, the Social Avoidance and Distress Scale, the Fear Questionnaire, a Global Social and Work Disability Scale, the Zung Self-Rated Anxiety Inventory, the Spielberger State/Trait Anxiety Inventory, and the Beck Depression Inventory.

C. Major Findings

There is a great deal of phenomenological and neurobiological overlap among the anxiety disorders. While current DSM-III-R criteria presuppose that social phobia and panic disorder are distinct conditions, few studies have investigated the diagnostic validity of this assumption. We examined whether patients with social phobia and patients with panic disorder, with or without agoraphobia, could be differentiated on the basis of demographic variables and response(s) to rating scales. Sixty-six patients with social phobia and 60 patients with panic disorder (42 with and 18 without agoraphobia) were studied. There was no significant difference in the ratio of men to women between the overall group of panic disorder versus social phobic patients. However, when the panic disorder patients are split into those with and without agoraphobia, there was a greater proportion of men (66.7%) in the panic disorder group without agoraphobia. In the panic disorder with agoraphobia group, the ratio of men to women (26.2% to 73.8%) was smaller than the ratio of men to women (36.4% to 63.6%) in the social phobic group. On all measures of social anxiety and avoidance and fears of negative evaluation, the social phobic patients consistently scored higher than the panic disorder patients. In contrast, panic disorder patients scored significantly higher than social phobic patients on measures of fear and avoidance of situations from which escape might be difficult in the event of a panic attack. We found patients with generalized social phobia to be remarkably different from discrete social phobia on a number of rating scales. This study (Gelernter et al., 1992) provides evidence for the diagnostic distinction between social phobia and panic disorder. This study provided little evidence for the separation of panic disorder with agoraphobia from panic disorder without agoraphobia.

II. Significance to Biomedical Research and Program of the Institute

Our findings support the current diagnostic separation of social phobia from panic disorder.

III. Proposed Course of Project

Future studies will examine patients with both social phobia and panic disorder in order to continue to describe features of the overlap syndrome. This is important because panic patients with "secondary" social phobia seem to respond to medications that fail to be effective in patients with "primary" social phobia.

Orthostatic Challenge Paradigm

A. Methods

Change in posture from supine to standing places a physiologic stress on an individual. There is a rapid increase in blood pressure and pulse mediated, in part, by release of norepinephrine (NE).

Patients with major depression have significantly greater increases in NE in response to standing compared to controls. Patients come to the clinic at 9 AM following an overnight fast and 72 hours on the low monoamine, low caffeine diet. An intravenous catheter is inserted in an antecubital vein and kept patent with a slow infusion of normal saline. After at least a 30 minute equilibration period, a blood sample is drawn for NE determination. The subject then stands for five minutes and a second sample is obtained for NE. Blood pressure (BP) and heart rate are measured at 75 second intervals.

B. Major Findings

14 patients with social phobia (SP), 14 normal control subjects, and 14 patients with panic disorder (PD) were investigated after orthostatic challenge. The SP patients exhibited supine and upright plasma NE levels that were significantly higher than the other two diagnostic groups. These observations suggest that the anxiety disorders may demonstrate differing patterns of autonomic dysfunction.

II. Significance to Biomedical Research and Program of the Institute

These results suggest that patients with panic disorder versus social phobia may have different patterns of autonomic dysfunction.

III. Proposed Course of Project

Projects using the orthostatic challenge method will be terminated.

Treatment Studies:

A. Methods

Under separate protocols, patients receive active medications and/or placebo in single- or double-blind fashion for at least 6 weeks. In selective studies, open treatment is offered to obtain preliminary information regarding response patterns and side-effect profiles. Standardized rating scales are employed to assess the patients under the different drug conditions at specified intervals.

B. Major Findings

Three studies are in their initial stages of development and one study has been recently completed (i.e. drug versus cognitive therapy).

Imipramine: Imipramine is effective in the treatment of panic and generalized anxiety disorder; however, it is unknown whether patients with social phobia benefit from this classic tricyclic compound. Although we had speculated that patients with social phobia would not respond to imipramine, preliminary evidence from an incomplete study suggest that a subgroup of social phobic patients with panic attacks may respond to imipramine pharmacotherapy.

Fluoxetine: There have been no reports of fluoxetine's efficacy in the treatment of social phobia. We openly treated 12 patients with social phobia with this serotonergic agent. Ten patients were treated with fluoxetine alone, while two were treated with fluoxetine in combination with another medication. Overall, 8 of 12 patients were moderately to markedly improved with fluoxetine treatment. Six of the ten patients treated with fluoxetine alone were moderately to markedly improved. These preliminary findings, which require confirmation with double-blind, placebo-controlled studies, suggest that fluoxetine may have a role in the treatment of selective groups of patients with social phobia.

Imipramine versus Phenelzine: We are currently conducting a study to directly compare the relative effectiveness of phenelzine versus imipramine in the treatment of social phobia. No data have been analyzed in this study.

Drug versus Cognitive Therapy: Sixty-five patients with social phobia were treated in a study that compared a cognitive behavioral group treatment program with pharmacotherapy with alprazolam, phenelzine, or pill-placebo plus instructions for self-directed exposure to phobic stimuli. All treatments were found to be effective, although patients who were treated with phenelzine were rated by clinicians as more improved on a measure of work and social disability than patients who were treated with alprazolam or placebo.

II. Significance to Biomedical Research and Program of the Institute

Overall, our findings suggest that different anxiety syndromes may preferentially respond to different pharmacological treatments. Our findings further suggest that phenelzine, a monoamine oxidase inhibitor antidepressant, may be the current drug of choice for patients with social phobia. Finally, our findings indicate that cognitive-behavioral therapy is quite effective in the treatment of social phobia.

III. Proposed Course of Project

The study investigating cognitive-behavioral versus drug therapies has been completed and will be terminated. The double-blind study of the direct comparison between imipramine versus phenelzine will probably be completed within the next 12-18 months. Because fluoxetine was effective in the treatment of social phobia, we treated a single case of elective mutism with fluoxetine. This young patient responded quite favorably to fluoxetine. Therefore, we have decided to investigate the phenomenology of elective mutism, as a possible variant of social phobia. We also plan to initiate drug trials with either fluoxetine or a MAO-inhibitor agent in the treatment of children with elective mutism.

Neuroendocrine Studies:A. Methods

Neuroendocrine studies of social phobia have been quite limited. We have investigated peripheral thyroid indices, and the behavioral and biochemical responses to α -2 adrenergic agents and caffeine in socially phobic patients.

B. Major Findings

In our comparisons of hypothalamic-pituitary-thyroid axis function in persons with social phobia and normal controls, the patients with social phobia had normal plasma levels of T_3 , T_4 , and thyroid-stimulating hormone (TSH). Social phobic patients also have a normal TSH response to TRH. The patients with social phobia also have normal levels of 24-hour urinary free cortisol and normal levels of basal and post-dexamethasone plasma cortisol. Relative to patients with panic disorder, therefore, patients with social phobia appear to have fewer neuroendocrine disturbances. We have found, however, an exaggerated pressor response to TRH in social phobic patients compared to normal controls and panic disorder patients. Finally, we have also found that social phobic patients have a blunted GH response to intravenous clonidine, but, as reported by an independent laboratory, a normal GH response to oral clonidine.

II. Significance to Biomedical Research and Program of the Institute

Alterations in noradrenergic neurotransmission or adrenergic-receptor function may mediate the pressor and GH responses to TRH. To the extent that this is true, the increased TRH-pressor in social phobia may reflect brain-specific alterations in noradrenergic function. Social phobia and panic disorder may be characterized by partial areas of overlapping dysfunction at different levels of the noradrenergic nervous system.

III. Proposed Course of Project

Future research will explore the mechanisms underlying these alterations in neuroendocrine function. A major focus of research will explore alterations in hypothalamic-GH-somatomedin function in social phobia.

Publications:

Black B, Tancer ME, Uhde TW. Fluoxetine for treatment of social phobia, J Clin Psychopharmacol, in press.

Black B, Uhde TW. Elective mutism as a variant of social phobia, J Am Acad Child Adolesc Psychiatry, in press.

Gelernter CS, Stein MB, Tancer ME, Uhde TW. An examination of syndromal validity and diagnostic subtypes in social phobia and panic disorder, J Clin Psychiatry 1992;53:23-7.

Gelernter CS, Uhde TW, Cimbalic P, Arnkoff DB, Vittone BJ, Tancer ME, Bartko J. Cognitive-behavioral and pharmacological treatments for social phobia: a preliminary study, Arch Gen Psychiatry 1991;48:938-45.

Rao SM, Devinsky O, Grafman J, Stein M, Usman M, Uhde TW, Theodore WH. Viscosity and social cohesion in temporal lobe epilepsy, J Neurol Neurosurg Psychiatry 1992;55:149-52.

Uhde TW, Tancer ME, Black B, Brown TM. Phenomenology and neurobiology of social phobia: comparison with panic disorder, J Clin Psychiatry 1991;52:31-40.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02519-03 BP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Patients with panic disorder: a comparison to social phobics, depressed patients and normal controls

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Thomas W. Uhde, M.D., Section Chief, BPB, NIMH

Albert Lin, Fogarty Associate, BPB, NIMH

Terry M. Brown, D.O., University of Georgia

Bruce Black, M.D., BPB, NIMH

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COOPERATING UNITS (if any)

University of Georgia

Biological Psychiatry Branch

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PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study examines the sleep architecture (polysomnography) of patients with panic disorder, social phobia, and depression, in comparison with that of normal controls. Continued analysis of such factors as movement time, REM latency, wakefulness after sleep onset, and sleep efficiency among the groups is an ongoing focus of research. The study of sleep panic attacks is a major focus of research for the Section. Evidence from the sleep laboratory continues to indicate that sleep panic attacks emerge from non-REM sleep -- typically either stage 2 or 3. On the nights when patients have had panic attacks, a prolonged REM latency has been noted, in contrast to the shortened REM latency of depression. End-tidal CO2 is being measured and measures of sleep apnea and nocturnal myoclonus are being employed. New methods of data analysis of sleep records are being developed such as recording number of K-complexes, spindles, and performing spectral analysis of data for between group comparisons. The Section is developing a methodology for quantifying vagal tone during sleep. Future studies will investigate the behavioral, biochemical, and neuroendocrine effects of chemical panicogenic agents administered during sleep in panic disorder patients and normal control subjects.

I. Project Description

A. Objectives

1. To determine if there are specific sleep architecture correlates of the anxiety and mood disorders.
2. To determine the physiological correlates of sleep panic attacks and to determine whether there are any substantial differences between those and other parasomnias such as night-terrors, nightmares, or non-specific arousals.

B. Methods Employed

Patients range in age from 15-70 and meet Research Diagnostic Criteria for panic disorder, social phobia, or major depression. Selective patients with other parasomnias also will be considered for participation in this study. Normal control subjects are age- and sex-matched with index patients.

Subjects are asked to stay in the sleep laboratory for polysomnographic study for 1-to-4 consecutive nights while medication-free. Some patients, if participating in treatment protocols, have polysomnographic studies both on and off of medications, to determine what effects the medications have on sleep architecture. Some subjects receive neuropeptides or drugs during sleep to assess the effects of these agents on sleep behavior and electroencephalography. All subjects receive standard mood and anxiety rating scales. Subjects also maintain a sleep diary prior to and during the sleep study. Pulse rates, blood pressure, vagal tone and selective biochemical indices are assessed during some phases of the study. Sleep panic attacks are recorded via an automated system developed by the SAAD.

C. Major Findings

The SAAD continues to find that sleep panic attacks emerge during non-REM sleep stages 2 or 3. Patients recall their sleep panic attacks. Physiological changes in heart rate and respiratory rate occur at, or slightly preceding, the time the patients wake up. Analysis of changes in end-tidal CO₂ is pending.

The sleep EEG of social phobic patients was not significantly different compared with age-matched normal control subjects.

II. Significance to Biomedical Research and Program of the Institute

Further elucidation of the differences between the sleep of patients with anxiety disorders from that of depressed patients and normal volunteers may be useful as a biological marker, as well as suggesting mechanisms for the role of sleep in these disorders.

Further study of sleep panic attacks together with comparisons with other parasomnias may provide a clearer differentiation between these disorders, and lead to more specific treatment of each of them.

III. Proposed Course of Project

Future work will concentrate on 1) increasing the pool of subjects from each category; 2) employing chemical models such as caffeine, pentagastrin, idazoxan, and CCK-4 to investigate the neurobiology of nonpanic and panic arousal states.

Publications:

Mellman TA, Uhde TW. Sleep of patients with panic disorder: Comparison with depression and normal controls. Sleep, in press.

Uhde TW. Sleep and the Anxiety Disorders. In Principles and Practice of Sleep Medicine, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02522-03 BP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Assessment of Family Functioning, Fear, and Panic Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

T.W. Uhde, M.D., Chief, Section on Anxiety and Affective Disorders, BPB, NIMH

B.S. Scupi, M.S.W., L.C.S.W., BPB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

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OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NIMH patients (hospitalized and outpatients) with diagnosed anxiety disorders (panic disorder, social phobia, generalized anxiety), affective disorders (major affective disorders) and normal volunteers are evaluated on psychological, interactional and developmental dimensions using self-administered questionnaire methodology. Attention is given to the role of family structure and functioning, and certain individual developmental experiences in the etiology and course of these diagnostic disorders. The National Institute of Mental Health Panic Disorder Questionnaire (NIMH-PQ) has been developed by the Section to assess special clinical and life-course of illness variables in patients with known panic disorder. This instrument has good validity on items that it shares with the SADS-L.

I. Project Description

A. Objectives

1. Project 1 was designed to learn more about whether: a) certain types of life events, family structure, quality of the parent-child relationship (care, over-protection, etc.), and types of fears predict treatment outcome.

2. Project 2 was designed to assess, in patients with known or suspected panic disorder, the phenomenology of panic, the temporal evolution of panic and related symptoms (panic attacks, agoraphobia, depression) and the course of illness.

B. Methods Employed

Project 1: Standardized questionnaires are administered to two patient groups (anxiety and affective disorder patients) and to normal controls, to gather relevant retrospective data.

Project 2: The National Institute of Mental Health Panic Disorder Questionnaire (NIMH PQ) is administered to patients with known or putative panic disorder.

C. Major Findings

Project 1: Data are being collected for this project and results have not been analyzed.

Project 2: Initial analysis of data generated in approximately 1300 patients with putative panic disorder indicate that this instrument has good reliability and validity across 16 similar or identical items on the NIMH-PQ and the SADS-L instruments. Several research teams throughout the world plan to use this instrument as part of their routine clinical evaluation of patients with panic disorder or to generate a data base for selective research hypotheses.

II. Proposed Course of Project

We will continue to investigate the phenomenology, early childhood experiences and longitudinal course of panic disorder. Our studies will focus on the phenomenology of panic attacks, type of panic attacks, psychosocial and biological triggers of panic. Areas of research focus include the investigation of panic attack duration as a predictor of illness severity and treatment response. Also, the NIMH-PQ will be utilized to evaluate the phenomenology, natural course, and co-morbid conditions associated with the sleep panic syndrome.

III. Significance to Biomedical Research and Program of the Institute

Anxiety disorders affect a large percentage of the population. As many as 12 million Americans may suffer from panic disorder at some point in their lifetime. Most of these individuals will experience significant impairment in functioning and productivity. This study will extend our knowledge of the phenomenology,

natural course of illness and early childhood expressions of panic disorder and provide new information regarding the poorly understood syndrome of sleep panic.

Publications:

Scupi BS, Maser JD, Uhde TW. The National Institute of Mental Health Panic Questionnaire (NIMH-PQ): An instrument for assessing clinical characteristics of panic disorder. J Nerv Ment Dis, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02540-02 BP

PERIOD COVERED
October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Neurobiology of Panic Disorder Humans and Nervous Pointer Dogs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients meeting DSM-III-R criteria for panic disorder and nervous pointer dogs, an animal model of panic disorder, are evaluated using chemical model strategies, neuroendocrine challenge techniques, and physiological and biochemical methodologies. Particular attention is given to the role of the noradrenergic, dopa-minergic, serotonergic, adenosinergic, and cholinergic neurotransmitter or neuro-modulatory systems in the pathogenesis of abnormal fear behaviors and regulation of hypothalamic-pituitary function. Patients with panic disorder were found to have diurnal changes in ratings of generalized anxiety, phobic anxiety and frequency of panic attacks and increased anxiogenic responses to m-CPP and pen-tagastrin. Both panic disorder humans and nervous pointer dogs have disturbances in hypothalamic-growth hormone function; in nervous dogs, this abnormality is associated with decreased levels of IGF-I in the plasma.

Other Personnel:

L. Malloy, BPB, NIMH

S. Slate, BPB, NIMH

I. Project Description

A. Objectives

1. Panic Disorder Humans: To improve our understanding of the neurobiology of patients with panic disorder, with and without agoraphobia.
2. Nervous Pointer Dogs: To ascertain the validity of nervous pointer dogs as a model of human anxiety disorders, particularly panic disorder and social phobia, and to document the neuroendocrine, physiological, and biochemical correlates of fear behaviors at different stages of development.

B. Methods Employed

1. Panic Disorder Humans: The Section investigates diurnal changes in anxious symptoms in panic disorder patients using 100 millimeter analog scales of generalized anxiety, phobic anxiety, and phobic avoidance.

The effects of meta-chlorophenylpiperazine (0.5 mg/kg), compared with caffeine (480 mg) and placebo, are tested in panic disorder patients.

Pentagastrin, a synthetic neuropeptide that binds to CCK-A and CCK-B receptors, has been used by our Section as a tool to investigate the role of the cholecystokinin system in the neurobiology of panic disorder.

The orthostatic challenge paradigm is used to investigate noradrenergic function in panic disorder patients. In this paradigm, subjects are asked to remain at bedrest for a minimum of 30 minutes, after which a baseline blood sample is obtained for the measurement of plasma NE. The subjects are then instructed to stand up, and to remain standing for exactly 5 minutes at which time a second blood sample for NE is drawn from a 3-way stop cock.

2. Nervous Pointer Dogs: The weight of the pituitary and adrenal glands, plasma levels of cortisol & ACTH, cerebrospinal levels of CRF and ACTH were measured in nervous and normal pointer dogs during nonstressful circumstances. Surgical removal of the brain was performed under sterile and anesthetic conditions. Brains were later removed and dissected while frozen using a punch technique. Samples are measured for NE, dopamine, and serotonin (as well as their metabolites) in both the nervous and normal line of dog. mRNA for growth hormone and somatostatin is also a focus of research.

The nervous and normal pointer dogs are now in the early phases of a study to investigating the relationship between velocity of growth and growth patterns and hypothalamic-pituitary-somatomedin function.

C. Major Findings

1. Panic Disorder Humans: The Section on Anxiety and Affective Disorders continues to find evidence for diurnal changes in ratings of generalized and phobic anxiety. Increased severity of symptoms and prominent diurnal changes are most evident in patients with a positive versus negative history of depression.

Panic disorder patients with a history of depression tend to have more frequent panic attacks in the morning or early afternoon.

In the m-CPP study, none of the patients experienced panic attacks after placebo administration. In contrast, we found that five of seven (70%) panic disorder patients had panic attacks after m-CPP and five of seven (70%) had panic attacks after caffeine. Four patients had panic attacks following both challenges, one had a panic attack only after m-CPP and one only after caffeine. Moreover, compared to placebo, the panic disorder patients had significant and equivalent increases in ratings of Zung Anxiety, NIMH anxiety, and NIMH global impairment after both m-CPP and caffeine. Both m-CPP and caffeine produced significant equivalent increases in plasma cortisol but only m-CPP produced significant increases in prolactin. These findings provide further evidence for serotonergic and adenosinergic dysfunction in the neurobiology of panic disorder.

Preliminary evidence suggests that panic disorder patients have an increased sensitivity compared with normal control subjects to the anxiogenic effects of pentagastrin. Increases in ratings of anxiety occur within 1-5 minutes after intravenous administration.

In the orthostatic challenge paradigm, patients with panic disorder were found to have an augmented HR response despite normal baseline and normal orthostatic-induced increases in NE. These observations suggest that alterations in vagal tone may be relevant to panic disorder patients under selective circumstances.

2. Nervous Pointer Dogs: The nervous and normal pointer dogs had similar plasma levels of ACTH and cortisol and CSF levels of CRH and ACTH. These findings parallel findings in panic disorder in that both nervous dogs and panic disorder humans have remarkably normal indices of hypothalamic-pituitary-adrenal axis function.

The nervous dogs had higher NE in the reticular formation and lower serotonin, and its metabolite 5-hydroxyindoleacetic acid, in the septal nuclei, indicating possible differences in noradrenergic and serotonergic function in nervous pointer dogs. There was a trend for lower HVA and DOPAC levels and a significantly lower DOPAC/DA ratio in the nervous dogs. Alterations in dopaminergic function may be relevant in the nervous line and will be the focus of future research.

The nervous pointer dogs have significantly lower total body weights, lower weight/height ratio and lower serum IGF-I levels compared with pointer dogs from the normal line. The best predictor of IGF-1 levels in the dogs, however, is the severity of fearful behaviors elicited by exposure to novel stimuli and humans.

II. Proposed Course of Project

The Section plans to initiate or further develop the caffeine, pentagastrin and yohimbine models of panic as a tool to study insomnia and sleep panic attacks. Moreover, we will utilize a new α -2 adrenergic antagonist, idazoxan, for the study of panic disorder during wake and sleep states.

The behavioral and neuroendocrine effects of caffeine, yohimbine and idazoxan also will be investigated in the nervous pointer dogs. Moreover, investigations are underway to study the relationship between the pharmacologic or chemical activation of hypothalamic-pituitary function and anatomic changes in pituitary and adrenal size in nervous versus normal pointer dogs. Studies are in their early phase of examining the mitral valves with echocardiography to assess the relationship between mitral valve disease and fear behaviors, if any, in dogs. Histopathological studies are also underway investigating receptor alterations in the cardiac muscles of the nervous and normal lines of dog.

In humans with anxiety disorders and nervous pointer dogs, investigations are will be conducted to evaluate the association between psychosocial stressors, age and gender on the onset of hypothalamic-growth hormone-somatomedin dysfunction.

III. Significance to Biomedical Research and Program of the Institute

Several epidemiological studies and the practical experience of family practitioners indicate that panic disorder attacks a large segment of the American population. This disabling condition strikes first at our youth and young adults. The long-term effects of panic disorder and other anxiety syndromes (as well as major life events and psychosocial trauma) on hypothalamic-growth hormone function are unknown. However, preliminary evidence from our laboratory suggest that problems with stature and emotional health may be quite significant. We intend to investigate biological correlates of anxiety and fear in the plasma and cerebrospinal fluid of panic disorder patients and nervous pointer dogs. These same studies, along with a wide-array of developing neuroendocrine, electrophysiological, brain imaging and chemical-model strategies, will be investigated in normal humans and in nervous pointer dogs.

An improved understanding of the psychosocial and biological aspects of both normal and pathological anxiety or fear are critically needed in order to develop new medications (with specific modes of action and fewer side-effects) for the treatment of panic and related anxiety disorders.

Publications:

Black B, Tancer ME, Uhde TW. Fluoxetine for treatment of social phobia. J Clin Psychopharmacol, in press.

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Gurguis GNM, Mefford IN, Uhde TW. Hypothalamic-pituitary-adrenocortical activity in panic disorder patients: relationship to plasma catecholamine metabolites. Biol Psychiatry 1991;30:502-6.

Mellman TA, Leverich GS, Hauser P, Kramlinger K, Post RM, Uhde TW. Axis II pathology in panic and affective disorders: Relationship to diagnosis, course of illness, and treatment response. *J Personality Disord* 1992;6:53-63.

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Uhde TW. Caffeine-induced anxiety: An ideal chemical model of panic disorder? In: Asnis GM, van Praag HM, eds. *Einstein monograph series in psychiatry*, in press.

Uhde TW, Bessette BB, Mellman TA. Anxiogenic and motor activity effects of caffeine in panic and major depressive disorders. *J Affective Disord*, in press.

Uhde TW, Malloy LC, Slate SO. Fearful behavior, body size and serum IGF-I levels in nervous and normal pointer dogs. *Pharmacol Biochem Behav*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00180-10 BP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychobiology and Treatment of Menstrually-Related Mood Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) David R. Rubinow, M.D., Chief, Section on Behavioral Endocrinology, BPB, NIMH P. Schmidt, T. Su, D. Rosenstein, BPB, NIMH; G. Merriam, L. Nieman, ERRB, NICHD,		
COOPERATING UNITS (if any) BPB, NIMH; ERRB, NICHD		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Behavioral Endocrinology		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The goals of this project are to detect and accurately describe <u>menstrually-related mood disorders</u> , explore their pathophysiology and response to pharmacological and environmental manipulation, and to document the relationship between reproductive <u>endocrine</u> change and disorders of mood as a way of further investigating the <u>neurobiology</u> of psychiatric illness. In the past year we have identified: 1) the ability of GnRH analogue (Lupron) to eliminate premenstrual syndrome during the second month of administration in some but not all patients; 2) the ability of progesterone to precipitate depression in 40% of women while on Lupron; 3) a significant decrease in mononuclear cell magnesium content and concentration as well as red blood cell magnesium in PMS patients compared with controls; 4) preliminary evidence of the efficacy of an infusion of magnesium during the luteal phase in premenstrual syndrome; 5) the therapeutic efficacy of fluoxetine in five of ten women with PMS; 6) the dissociation of the switch out of PMS from menses in two of four women with artificially prolonged luteal phases.		

I. Project Description

A. Objectives

This project has as its main intent the selection of subjects with carefully documented menstrual cycle related mood disorders who can then undergo psychological and biological evaluation as well as participate in double-blind, placebo-controlled trials of several treatment modalities.

B. Methods Employed

1. Subjects

- a. Subjects are self- and physician-referred women between the ages of 18 and 55 who meet study criteria as described in detail in Project Z01 MH 00180-03 BP.
- b. Controls for this study include women with no complaints nor evidence of menstrually-related mood disorder, who are without primary psychiatric illness, and women who have complaints of, but no visual analogue scale evidence of, menstrually-related mood changes.

2. Procedures

Phase 1. An extensive screening phase that has been described in detail in Project Z01 MH 00180-02 BP.

Phase 2. This is an intensive psychobiological evaluation phase for patients meeting entry criteria for the study.

a) Patients are given a thorough physical and laboratory examination in order to rule out the presence of unknown medical illness.

b) Ongoing studies of longitudinally obtained basal and stimulated hormonal levels have been previously described in Project Z01 MH 00180-03 BP. In addition, we are performing the following studies:

- 1) Stimulation/dynamic endocrine challenges during the follicular phases.
 - A) Gonadotropin releasing hormone infusions in collaboration with Dr. P. Schmidt.
 - B) m-CPP infusions in collaboration with Dr. T. Su.
- 2) Menstrual cycle manipulation studies.
 - A) Luteal phase extension with progesterone suppositories (Drs. P. Schmidt and G. Merriam).
 - B) Elimination of ovarian cyclicity with gonadotropin releasing hormone agonist (Drs. P. Schmidt and L. Nieman).

Phase 3. Multi-modality treatment phase for patients who have completed Phase 2. Double-blind, placebo controlled crossover evaluations of fluoxetine are currently being conducted. Rationales for the selection of these particular compounds have been previously described.

C. Findings

Five patients finished and four additional patients entered a study in which gonadotropin releasing hormone agonist (Lupron) is administered to suppress cyclicity in women with premenstrual syndrome, following which the gonadal ster-

oids progesterone and estrogen are administered in order to sequentially replace the main reproductive endocrine components of the menstrual cycle. Preliminary data suggests that Lupron eliminates the premenstrual syndrome in some but not all women, with recurrent (albeit, not cyclic) mood disturbances persisting in two of five patients. Additionally, re-exposure to progesterone precipitated prominent symptoms of depression and anxiety in two of five women studied. Other findings include the predictable loss of sexual interest in patients on Lupron, Lupron-associated memory impairment (analysis pending), and the responsiveness of Lupron-induced hot flushes to both estrogen and progesterone. Four patients have entered a study in which the luteal phase is prolonged by one week by means of progesterone suppositories. In this study to date, we have observed the offset of premenstrual syndrome symptoms both one week prior to and coincident with the delayed menses. These data, in combination with our data from the RU 486 study, demonstrate the inconsistent nature of the relationship between the luteal phase and the premenstrual syndrome state. The Lupron and luteal phase extension study promises to significantly increase our understanding of the linkage of mood state disorders to changes in gonadal steroids. In addition, the performance of the Lupron study in patients and controls will identify the role of gonadal steroids in circadian regulation, cerebral blood flow, and salivary flow.

In addition to a decrease in red blood cell magnesium in patient with PMS compared with controls, we have demonstrated a diagnosis-related decrease in mononuclear blood cell magnesium content and concentration (collaborator D. Rosenstein). These findings were demonstrable only when the sampling times were correctly assigned to menstrual cycle phase, not menstrual cycle day. In order to determine the possible significance of these findings, we have initiated an acute, placebo-controlled infusion of magnesium during the luteal phase in PMS patients and controls. While it does not appear that patients with premenstrual syndrome have a magnesium deficiency (as determined by magnesium retention), preliminary data suggest an acute and sustained therapeutic effect of magnesium infusion in premenstrual syndrome.

Finally, ten patients and ten controls have undergone m-CPP infusions during the luteal and follicular phases, with the patients subsequently entering a double-blind, placebo-controlled trial of fluoxetine. Five of the ten patients studied appeared to respond to fluoxetine with significant reduction or elimination of their PMS symptoms; the ability of response to m-CPP to predict response to fluoxetine awaits analysis of the m-CPP data.

D. Proposed Course of Project

We will continue to employ the Lupron and luteal phase extension protocols to attempt to characterize the role of gonadal steroids in premenstrual syndrome. Additionally, we will employ the Lupron protocol to determine the effect of gonadal steroids on cerebral blood flow, neuropsychological function, circadian regulation, and salivary flow in normal volunteers. We intend to characterize the switch in patients with premenstrual syndrome both phenomenologically and endocrinologically, employing frequent mood ratings and salivary cortisol measures. Finally, we have initiated a study of brief recurrent depressed patients as a control group for patients with menstrual cycle phase-dependent depressions. Studies of the therapeutic efficacy of fluoxetine and acute magnesium administration will be continued.

E. Significance to Biomedical Research and the Program of the Institute

This project will provide information that will be of immediate clinical relevance to those who suffer with menstrual cycle-related mood disorders and will further our understanding of the complex relationship between endocrine (particularly reproductive endocrine) system activity and mood and behavior.

Publications

Rubinow DR, Schmidt PJ. Models for the development and expression of symptoms in late-luteal phase dysphoric disorder. In: Blumenthal S, Haseltine F, Rubinow DR, eds. Late luteal phase dysphoric disorder: new research directions. Washington DC: American Psychiatric Press, in press.

Schmidt PJ, Rubinow DR. Parallels between PMS and psychiatric illnesses. In: Smith S, Schiff I, eds. Modern management of PMS. New York: W.W. Norton & Company, 1991, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00181-09 BP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Hormonal Studies of Affective Disorder		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) David R. Rubinow, M.D., Chief, Section on Behavioral Endocrinology, BPB, Dr. P. Lewitt, Lafayette Clinic; Dr. M. Demitrack, University of Michigan Medical Center; Dr. T. Su, BPB, NIMH, Dr. M. Linnoila, NIAAA,		
COOPERATING UNITS (if any) BPB, NIMH; Lafayette Clinic, Detroit, MI; NIAAA		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Behavioral Endocrinology		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Studies of <u>somatostatin</u> and <u>beta endorphin</u> in relation to affective and other <u>neuropsychiatric</u> disorders have continued. Findings consist of: A) <u>Somatostatin</u> - No diagnostic group-related differences were noted in CSF somatostatin in 57 violent offenders compared with 27 controls. Thirty-hour CSF studies showed a progressive increase in CSF somatostatin superimposed upon a diurnal rhythm in 14 patients. In a preliminary analysis, no diagnostic group-related differences were noted in CSF somatostatin in patients with Parkinson's dementia compared with controls. Similarly, no differences in CSF somatostatin were observed in patients with Sydenham's Chorea and controls. B) <u>Beta endorphin</u> - A significant inverse relationship was observed between CSF beta endorphin and measures of dissociation, depression, and anxiety in patients with eating disorders.		

I. Project Description

A. Objectives

The goal of this project is to study neuroendocrine and immune soluble products in patients with neuropsychiatric disorders in order to expand our understanding of the mechanisms and significance of reported abnormalities in somatostatin and immune system activity in affective illness.

B. Methods Employed

1. Subjects

a. Subjects include patients on NIMH clinical units meeting criteria for major depressive disorder, Alzheimer's disease, Huntington's dementia, Parkinson's dementia, multiple sclerosis, bulimia, anorexia nervosa, adults with obsessive-compulsive disorder.

2. Procedures

Lumbar punctures are performed to obtain CSF samples for somatostatin, CRF, and other related CNS peptides/neurotransmitters. Brain slices in animals are punched and assayed for somatostatin content. Whole brains are dissected and marker radioactivity measured in animal lymphokine studies. Animals are cannulated to measure blood hormone levels following IL-2 administration. Rat pituitary glands are cultured to measure stimulating effects of IL-2.

II. Findings

A. Somatostatin - CSF studies have continued in a variety of neuropsychiatric populations. Forty-five patients with Alzheimer's disease and 12 age-matched controls have been studied as part of the longitudinal effort to determine the relationship between CSF somatostatin and clinical deterioration in Alzheimer's disease. Fifty-seven violent offenders were compared with 27 controls, with no diagnostic group-related differences observed. A 30 hour CSF study was performed in collaboration with M. Kling and revealed progressive elevation of sample concentrations over time, perhaps suggestive of an effect of the sampling procedure on CSF neuropeptide concentrations. CSF somatostatin has also been measured in patients with Sydenham's Chorea and controls (collaborator S. Swedo) as well as in patients with Eosinophilia Myalgia Syndrome (EMS) and controls (collaborator M. DeBellis).

B. Beta Endorphin - CSF beta endorphin has been measured in patients with EMS and those with affective disorder during treatment with idazoxan or fluvoxamine (collaborator M. DeBellis). A significant inverse relationship was observed between CSF beta endorphin and measures of dissociation, depression, and anxiety in patients with eating disorders (collaborator M. Demitrack). Additionally, we have been measuring beta endorphin as the secretory outcome measure of IL-2 stimulation in AtT20 cell cultures.

III. Proposed Course of Project

We hope to: 1) expand our investigation of circadian salivary cortisol measures in depressed patients; 2) develop assay techniques to permit evaluation of

the contribution of somatostatin fragments to the total syndromal alterations in CSF somatostatin; 3) continue cell culture studies of the time-dependent sensitized endocrine response to IL-2, and, 4) measure somatostatin longitudinally in patients with Alzheimer's disease to determine whether the course of the disorder is reflected in continued decreases in CSF SRIF levels.

IV. Significance to Mental Health and the Program of the Institute

Depression-related dysregulation of somatostatin and cortisol may provide a window into the central neurochemical lesions responsible for depression. Further, specific behavioral or psychological disturbances (e.g. cognitive impairment or cortisol dysregulation) may be products of abnormal neuroendocrine activity. It may prove to be the case that depression-related reductions in somatostatin are mechanistically relevant to depression-related disturbances in hypothalamic-pituitary-adrenal activity, the most commonly reported biological abnormality in depression. Determination of the mechanisms of the profound behavioral and cognition altering effects of interleukin-2 would fill a major gap in our knowledge of the ways in which the immune system can regulate CNS activity. Further study may not only enhance our knowledge of the neurobiology of depression but may, as well, more generally inform us about the relationship between hormones and human behavior.

Publications

Martin PR, Adinoff B, Eckardt MJ, Rubinow DR, Lane EA. Fluvoxamine treatment of alcoholic amnesic disorder [Letter to the Editor]. Arch Gen Psychiatry, in press.

Molchan SE, Lawlor BA, Hill JL, Martinez RA, Davis CL, Mellow AM, Rubinow DR. CSF monoamine metabolites and somatostatin in Alzheimer's disease and major depression. Biol Psychiatry 1992;29:1110-1118.

Rubinow DR, Davis CL, Post RM. Somatostatin. In: Nemeroff C, ed. Neuropeptides in psychiatry. Washington DC: American Psychiatric Press, in press.

Rubinow DR, Davis CL, Post RM. Somatostatin in neuropsychiatric disorders. In: Weil C, ed. Basic and clinical aspects of neuroscience, vol 4. Basel: Sandoz Pharma LTD, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00182-09 BP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Behavioral Medicine		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) David R. Rubinow, M.D., Chief, Section on Behavioral Endocrinology, BPB, NIMH Dr. Peter Schmidt, BPB, NIMH Dr. Daniel Longo, Frederick Cancer Research Facility, NCI Dr. Christine Ollo, BPB, NIMH		
COOPERATING UNITS (if any) BPB, NIMH; FCRF, DCBDC, NCI; MN, NINDS; DEB, NICHD, University of Maryland		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Behavioral Endocrinology		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Six protocols are currently active and conducted out of the Consultation-Liaison Service-based <u>behavioral medicine</u> research program. These protocols examine the phenomenology and biological correlates of illness or treatment-induced mood, behavioral, and cognitive changes. The protocols address such areas as: a) the effects of previous psychiatric history on the psychiatric morbidity associated with certain diseases and their treatment; b) the psychiatric phenomenology of certain diseases and their treatment; c) the treatment response characteristics of psychiatric disorders associated with medical diseases or their treatment; d) biochemical factors that may serve as predictive diagnostic markers for illness or for treatment-associated mood/behavioral or cognitive syndromes; e) the effects of mood state alterations on <u>immunologic function</u> . Significant findings to date include demonstration of the following: 1) significant reduction in thyroid and reproductive axis activity during anabolic steroid administration; 2) significant increase in IL-2 and discoordination of IL-1 and IL-2 secretion by anabolic steroids; 3) evidence of significant cognitive impairment and negative affective symptoms in association with blunted endocrine response to anabolic steroids, with significant positive affective symptoms associated with more robust hormonal responses to anabolic steroids; 4) preliminary evidence of the antidepressant efficacy of thyroid hormone (T ₄) in patients with central hypothyroidism.		

Other Professional Personnel (Continued)

Dr. Jordan Grafman, Medical Neurology Branch, NINDS
Dr. Louis Vandermolén, Frederick Cancer Research Facility, NCI
Dr. Larry Tamarkin, University of Maryland
Dr. Donald Rosenstein, BPB, NIMH
Dr. Lynette Nieman, Developmental Endocrinology Branch, NICHD
Dr. Maria Turner, DCBDC, NCI

I. Project Description

A. Objectives

This project has as its main intent the identification of mood and cognitive symptoms that appear in the context of specific medical illnesses and their treatment, determination of the relationship between these symptoms and both the primary medical disorder and prior psychiatric history, and utilization of the occurrence of these symptoms in a medical context as models for the occurrence of similar symptoms in a primarily psychiatric context.

Protocols

Active:

- 1) Neuropsychiatric Effects of Anabolic Steroid Administration (collaborators: T. Su and D. Pickar)
- 2) Pudendal Neuralgia: A Therapeutic Trial with Desipramine and Investigations into Possible Etiologies (collaborator: M. Turner)
- 3) The Neuropsychiatric Aspects of Central Hypothyroidism and Their Response to Thyroid Hormone Supplementation (collaborators: L. Nieman, C. Ollo, D. Rosenstein)
- 4) Conditioned Immunosuppression and Immunoenhancement in Cancer Patients (collaborators: L. Vandermolen, L. Tamarkin, D. Longo, P. Schmidt).
- 5) The Effects of Psychostimulants on Depression in the Medically Ill (collaborator: D. Rosenstein).
- 6) Longitudinal Evaluation of CSF Chemistry and Cognitive Function in Seropositive Patients (collaborator: J. Grafman)

B. Methods Employed

1. Subjects

a. Subjects are NIH patients who are referred for participation in these protocols by collaborators from the Institute responsible for the primary care and treatment of these patients.

b. Controls for the individual studies are selected in a way that allows for stratification of populations with respect to the relevant variables under study.

2. Procedures

a. Psychiatric Diagnostic Evaluation

The primary methodology employed is that of evaluating the psychiatric history of all subjects and their families utilizing a semistructured psychiatric interview, the Schedule for Affective Disorders and Schizophrenia (SADS-L), which provides information from which an RDC diagnosis can be made.

b. Longitudinal Evaluation

Most studies utilize a "self as own control" design employing longitudinal assessment of mood ratings, physical symptoms, and cognitive performance. Detailed description of methodologies employed can be found in Project # Z01 MH 00182-02 BP.

c. Laboratory Assessment

Urine, blood samples, and CSF are collected in order to permit evaluation of those biological substances believed to be related to the development of affective or cognitive disturbances.

3. Findings

1) Anabolic steroids

Ratings of AS-induced distractibility were significantly correlated with increased plasma cortisol, plasma DHEA, and CSF ACTH as well as with blunted AS-induced testosterone suppression. Blunted effects of AS on reproductive hormones (testosterone, DHT, estradiol, SHBG, LH) were associated with increased cognitive impairment and negative (distressing) symptoms as well as with decreased positive symptoms (e.g., energy, confidence) during high dose AS administration. Finally, AS produced significant increases in IL-2 with decreases in IL-1, providing some of the first evidence of their discoordinated secretion. These data may help identify some of the biological mechanisms underlying both the adverse behavioral and health consequences of AS.

C. Proposed Course of Project

The studies noted above will be continued until adequate numbers of subjects are obtained. The effects of acute anabolic steroid administration on cerebral blood flow will be identified. A study is planned to investigate the relationship between acute gonadal steroid withdrawal and behavioral change in women and men treated with Lupron for cancer (breast cancer and prostatic cancer, respectively). A longitudinal study of CSF peptides and cognition in seropositive military personnel is being conducted in collaboration with Dr. J. Grafman. Protocols to investigate the efficacy of stimulants in the treatment of depression in the medically ill and to investigate the possible existence of conditioned immunosuppression and immunoenhancement in cancer patients will be continued.

D. Significance to Biomedical Research and the Program of the Institute

The studies in this project are hypothesis-generating as well as hypothesis-testing. Thus, they should not only help to expand the behavioral phenomenology of many medical disorders, but should, as well, suggest optimal studies for the application of modern neuroscientific techniques to disorders of regulation of mood and cognition. Detection of conditioned immunosuppression in patients should have profound effects on both our understanding and treatment of many medical disorders. Identification of markers for the acquisition and progression of HIV-related central nervous system dysfunction should greatly advance our understanding of the etiology and consequences of AIDS-related and possibly HIV-related dementias. Identification of the biological predictors of adverse response to anabolic steroids may help avoid some of the morbidity attendant to the use of these medications, as well as clarify the degree to which these compounds are behaviorally toxic. The utilization of medical disorders as models for the development of mood and cognitive disturbances in the context of biological dysregulation should clarify the meaning of those biological alterations already observed in psychiatric disorders.

Publications

Paciotti GF, Baron DA, Licinio J, Tamarkin L, Wong M-L, Gold PW, Altemus ME, Rubinow DR. Novel enzyme immunoassays for the detection of the cytokines interleukin 1 and interleukin 2 in the circulation of normal subjects: 24-hour profiles. *Prog Neuroendocrinol Immunol* 1982;5:21-30.

Peck GL, DiGiovanna JJ, Rubinow DR. Acute depression from isotretinoin: reply. J Am Acad Dermatol 1991;25:132.

Rubinow DR, Post RM. Impaired recognition of affect in facial expression in depressed patients. Biol Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02537-03 BP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychobiology and Treatment of Peri-Menopausal Mood Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) David R. Rubinow, M.D., Chief, Section on Behavioral Endocrinology, BPB, NIMH P. Schmidt, BPB, NIMH T. Su, BPB, NIMH L. Nieman, ERRB, NICHD P. Gindoff, George Washington University J. Murphy, OCD, NIMH		
COOPERATING UNITS (if any) BPB, NIMH; ERRB, NICHD; George Washington University		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Behavioral Endocrinology		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The goals of this project are to detect and accurately describe peri-menopausal mood disorders, explore their pathophysiology and response to pharmacological and environmental manipulation, and to document the relationship between reproductive endocrine change and disorders of mood as a way of further investigating the neurobiology of psychiatric illness. Findings to date include: 1) preliminary evidence of the therapeutic efficacy of estrogen in ten peri-menopausal women with non-hot flush-related depressions; 2) absence of evidence of abnormal response to TRH stimulation in peri-menopausal depressed women compared with controls; 3) preliminary evidence of the spontaneous reversal of ovarian insensitivity-related depression and hot flushes in peri-menopausal women; 4) significant clomiphene citrate-induced elevations of FSH and decreases of estradiol in women with irregular menstrual cycles compared with age-matched, regularly cycling controls.</p>		

I. Project Description

A. Objectives

This project has as its main intent the selection of subjects with carefully documented peri-climacteric mood changes who can then undergo psychological and biological evaluation, as well as participate in double-blind, placebo-controlled trials of psychotropic and hormonal treatment modalities.

B. Methods Employed

1. Subjects

a. Subjects are self- and physician-referred women between the ages of 40 and 60 who meet study criteria as described in detail in project Z01 MH 02537-01 BP and are in the climacteric or within six months of menopause. Subjects will consist of those women with recent onset mood disorders with or without accompanying hot flushes, women with hot flushes without mood disorders, and women with no climacteric-related symptomatology who will be followed longitudinally through the climacteric and menopause.

b. Controls for this study include women with no complaints nor evidence of climacteric-related mood or somatic disorder and who are without primary psychiatric illness.

2. Procedures

Phase 1. An extensive screening phase has been detailed in project Z01 MH 02537-01 BP.

Phase 2. This is an intensive psychobiological evaluation phase for patients meeting entry criteria for the study.

a) Patients are given a thorough physical and laboratory examination in order to rule out the presence of unknown medical illness.

b) Basal and stimulated endocrine function is measured as follows:

1) Serial FSH levels.

2) TRH and GnRH stimulation tests (collaborator:

P. Schmidt).

3) Clomid stimulation studies (collaborator: P. Gindoff).

c) Patients with hot flushes are evaluated with physiologic monitoring at baseline and following m-CPP infusions.

Phase 3. Patients experiencing significant mood symptoms are invited to participate in an estrogen replacement protocol to determine, in a double-blind, placebo-controlled fashion, the efficacy of estradiol in climacteric-related mood disturbances (collaborators: P. Schmidt, L. Nieman, J. Murphy).

C. Findings

Ten women have completed and 13 entered a double-blind, placebo-controlled trial of estrogen in the treatment of peri-menopausal depression in the absence of hot flushes. This patient group was selected in order to avoid confusion of a primary effect of estrogen on mood with an effect secondary to estrogen-treated hot flushes. Thus far, a significant beneficial effect of

estrogen on affective symptoms (sadness, tearfulness, worry) as well as sleep disturbance and fatigue has been identified. The effects of estrogen on cognitive function await analysis of the collected data. Twenty-four hour physiologic monitoring has been performed in these patients in order to identify the effects of estrogen on thermo-regulation in patients who do not complain of hot flushes. Similar monitoring has been performed in four patients with hot flushes and four peri-menopausal controls. TRH infusions in seven patients with peri-menopausal depression and seven controls have revealed no abnormality of response. Oral m-CPP challenge tests are being performed to evaluate the endocrine and thermal response to a serotonergic agonist in peri-menopausal women. Performance of the clomiphene citrate challenge test demonstrated a significantly increased FSH and significantly decreased estrogen response in women with irregular menstrual cycles compared with age-matched normal cycling women. These changes in women with new onset irregular cycles are particularly interesting given our observation of the reversal of depression and hot flushes and normalization of FSH levels in peri-menopausal women who spontaneously resume normal cycling.

D. Proposed Course of Project

With a group of well defined patients we hope to explore the natural course of climacteric-related mood disorders as well as their phenomenology and biologic correlates in relation to treatment response. The prevalence and characteristics of mood disorders arising during the peri-menopausal period will be determined with longitudinal as well as cross-sectional studies. Further, the proximity of behavioral change to alterations in reproductive endocrine activity will be closely examined. The neurochemical determinants of thermoregulatory disturbances, as well as the relationship of these disturbances to alterations in mood, will be investigated with administration of pharmacologic probes (e.g. m-CPP) of neurochemical systems believed critical for thermoregulation. Finally, the efficacy of hormonal replacement in the treatment of peri-menopausal mood disturbances will be determined.

E. Significance to Biomedical Research and the Program of the Institute

These longitudinal studies offer the promise of confirming or disconfirming centuries old observations of mood changes occurring during the perimenopause. Additionally, this project provides an opportunity to further investigate the role of changes in reproductive endocrinology in behavioral regulation.

Publications

Schmidt PJ, Rubinow DR. Menopausal mood disorders: past and future research strategies. In: Blumenthal S, Haseltine F, Rubinow DR, eds. Late luteal phase dysphoric disorder: new research directions. Washington, DC: American Psychiatric Press, Inc, in press.

Schmidt PJ, Rubinow DR. Menopausal-related mood disorders: a justification for further study. Am J Psychiatry 1991;148:844-852.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 MH 02527-03 BP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Sensitization

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Susan R.B. Weiss, Ph.D., Chief, Unit of Behavioral Biology, BPB, NIMH

Robert M. Post, M.D., Chief, BPB, NIMH

Agu Pert, Ph.D., BPB, NIMH

D. Nigel Thomas, Ph.D., MacArthur Fellow, BPB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychobiology

INSTITUTE AND LOCATION

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TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The overall objectives of this project are to study the phenomenology, and the biochemical and neuroanatomical substrates of behavioral sensitization. This model is used to study the evolution of behavioral pathology and the role of conditioning or context-dependency in such behaviors. Environmental and pharmacological interventions are investigated in an attempt to alter the course of sensitization development and expression. Significant findings to date include demonstration of the following: 1) a novel two day cocaine sensitization paradigm which is context-dependent; 2) a second sensitization paradigm, using a three day pretreatment regimen, which is also context-dependent and is more long-lasting than the above paradigm; 3) the importance of intact dopamine function for the development, but not expression of context-dependent sensitization; 4) a role for the amygdala and nucleus accumbens in this sensitization; 5) cross sensitization between cocaine and the NMDA antagonist MK-801, and between cocaine and procaine (a local anesthetic that is self-administered by primates), but not between cocaine and lidocaine (which is not self-administered) or cocaine and caffeine.

I. Project Description

A. Objectives

The project on behavioral sensitization to cocaine has as its main intent the characterization of context-dependent or conditioned cocaine sensitization, and an examination of the functional contribution of specific neurotransmitters, peptides and neuroanatomical structures. Conditioning factors are often overlooked in the treatment of human drug abuse, but can be a major component of a drug's subjective effects, and the withdrawal symptoms or craving associated with drug discontinuation. As such, an understanding of the biological mechanisms involved in this phenomenon could be of great theoretical and therapeutic utility.

B. Methods Employed

1. Subjects: Male Sprague Dawley rats, 200-250 g.

2. Procedures:

a. A two day paradigm has been developed in which animals are administered a high dose of cocaine (40 mg/kg) or saline on day 1, in the test chamber, and a low dose of cocaine (10 mg/kg) on day 2. The measure of behavioral sensitization is an increased locomotor activity or stereotypy response to the challenge dose of cocaine on day 2. To elucidate the importance of conditioning or context dependency in this paradigm, a separate group of rats is administered the same high dose of cocaine (40 mg/kg) on day 1, but in an environment other than the test chamber and then given the low-dose cocaine challenge on day 2. This paradigm results in a sensitized or enhanced response to the low dose of cocaine only in the animals previously treated with cocaine in the test chamber.

b. A number of pharmacological agents have been administered in addition to cocaine on day 1 to attempt to block the development of cocaine sensitization, or on day 2 to block the expression of sensitization.

c. Other agents were examined for their ability to cross sensitize with cocaine. If this occurs, common mechanisms of action could be further studied.

d. Electrolytic or 6-OHDA lesions were made of the amygdala, nucleus accumbens, dorsal hippocampus, ventral hippocampus, and cerebellum. The role of these structures in the development of sensitization was studied.

e. A second sensitization paradigm was developed in which cocaine (40 mg/kg) or saline are repeatedly administered for three days followed by a low dose challenge of cocaine (10 mg/kg) on day 4 and again ten days later. This paradigm has the advantage of producing a longer lasting context-dependent sensitization, a less robust context-independent sensitization (i.e., in animals treated with equivalent doses of cocaine, but in their home cage), and could thus be used to examine the role of certain brain structures in the expression of sensitization or in context-independent sensitization.

3. Findings

1. The two-day behavioral sensitization paradigm is totally context-dependent as there are no differences in the response to cocaine on day 2 (10 mg/kg), between the group that received only saline on day 1 and the group that received cocaine in their home cage. Moreover, the sensitized response, like other forms of conditioned or learned responses, shows a generalization gradient, such that sensitization is greater in animals administered cocaine in an

environment similar to the test chamber as compared to environments of lesser similarity.

2. In this paradigm, haloperidol was found to block the development but not the expression of cocaine sensitization. To the extent that sensitization phenomena are a component of the course of illness in schizophrenia, this paradigm represents a potential model of neuroleptic non-responsiveness in schizophrenic patients. In such cases, treatments that can effect the expression of sensitization may be of therapeutic value. Clonidine and diazepam block both aspects of sensitization and carbamazepine given chronically or acutely affects neither the development nor the expression of sensitization.

3. A number of studies demonstrate an interaction between stress and the effects of stimulants, including cross sensitization between these phenomena. We have also shown that the stress hormone CRH markedly potentiates cocaine kindling (Z01 MH 02528-01 BP). For these reasons we examined whether the CRH antagonist (alpha-helical CRH) could block the development of cocaine sensitization as it does cross sensitization between amphetamine and stress, and also acquisition of conditioned behavioral suppression. No effect of the antagonist was found for context-dependent cocaine sensitization, suggesting that removing this component of the stress response (i.e., CRH) was not sufficient to block context-dependent cocaine sensitization.

4. Lesions of the amygdala (electrolytic or 6-OHDA) and lesions of the nucleus accumbens (6-OHDA) block the development of cocaine sensitization (while the day 1 cocaine-induced hyperactivity was unaffected). Lesions of the hippocampus or cerebellum were without effect in this paradigm.

5. Lesions of the amygdala (electrolytic) do not block context dependent sensitization using the three day repeated cocaine (40 mg/kg) paradigm. This effect was replicated using rats subjected to both the two day and the four day paradigm, with rechallenge at 10 days. In the two day paradigm, the amygdala lesion blocked the sensitization, but, subsequently, in the four day paradigm and during the rechallenge, the lesion became ineffective. These data suggest that different anatomical substrates become involved with the repetition of events, as occurred in this paradigm. Attempts to evaluate the importance of striatal mechanisms in this paradigm are underway.

C. Proposed Course of Project

Other agents will be tested for their importance in the development or expression of sensitization, e.g., serotonin reuptake blockers and antagonists, calcium channel antagonists. Of particular interest would be agents effective against the expression of sensitization as they may indicate preferred strategies for dealing with either drug abuse or treatment -refractory schizophrenics.

The lesion studies will also continue with attempts to interfere with context-independent sensitization, the expression of sensitization, as well as sensitization in the three day paradigm. Lesions of the caudate nucleus or its efferent connections will be attempted to examine the latter. Lesions within the amygdala, particularly in the central nucleus, will also be attempted since this nucleus appears to be important in a number of other conditioning paradigms.

Studies are underway (with Drs. J. Rosen, M. Clark, and M. Smith) to determine whether the context-dependent cocaine sensitization is associated with a

unique pattern of induction of transcription factors such as Fos, Jun, and the FRAs, as well as neuropeptide mRNAs. Comparisons will be made with context-independent exposure to cocaine as well as with repeated administration of other agents not typically associated with a sensitization response, e.g., caffeine.

D. Significance to Biomedical Research and the Program of the Institute

These studies are important in the understanding of basic mechanisms of conditioned drug effects. New techniques are now available to determine the effects of drug administration on gene expression, which may ultimately lead to novel insights regarding long-term changes in behavior relating to drug abuse and dependence. These models may also provide data relevant to psychiatric illnesses such as mania, and schizophrenia. The change in responsivity to a drug that results from its repeated administration, and the associative learning that occurs with drug administration are of importance to our understanding of subjective drug effects, as well as withdrawal and craving symptomatology following drug abstinence. Our data suggest that treatment strategies for drug abuse should include principles of "unlearning" or "deconditioning" the drug-associated stimuli, along with pharmacological manipulations that can alter or inhibit an abused drug's effects.

Publications:

Post RM, Weiss SRB, Fontana D, Pert A. Conditioned sensitization to the psychomotor stimulant cocaine. *Neurobiology of Drug Addiction* 1992;654:386-399.

Post RM, Weiss SRB, Pert A. Sensitization and kindling effects of chronic cocaine administration. In: Lakoski JM et al, eds. *Cocaine: pharmacology, physiology, and clinical strategies*. Caldwell NJ: Telford Press, 1991;115-61.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02528-0.3 BP

PERIOD COVERED
October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Pharmacological Kindling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Susan R.B. Weiss, Ph.D., Senior Staff Fellow, BPB, NIMH

Robert M. Post, M.D., Chief, BPB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Psychobiology

INSTITUTE AND LOCATION
NIMH, Bethesda, Md.

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to study and manipulate the course of development of pharmacologically kindled seizures using the local anesthetics lidocaine and cocaine. In addition to seizures, other behavioral abnormalities are studied, including behavioral stereotypies with cocaine, aggression with lidocaine, and mortality related to cocaine seizures. The kindling effects of cocaine are likely related to its local anesthetic properties, as similar effects are seen with lidocaine but not with other psychomotor stimulants. The importance of local anesthetic mechanisms in human cocaine abuse remains to be determined, but aspects of cocaine-related panic disorder (which is reported by 50% of cocaine users calling a cocaine hotline) resemble kindled seizure development and thus provide an intriguing clinical-basic research overlap. Significant findings of this project include the demonstration that 1) the development of local anesthetic kindled seizures and their associated lethality can be prevented with chronic, but not acute or repeated acute, carbamazepine treatment; 2) the local anesthetic kindled seizure model may offer a novel approach to examining carbamazepine's mechanisms of action in affective illness, which also requires chronic treatment; 3) the following systems have been ruled out as being necessary to carbamazepine's anticonvulsant effects in this seizure model: alpha-2-adrenergic receptors, peripheral-type benzodiazepine receptors, serotonin and somatostatin; 4) the stress related peptide corticotropin releasing hormone (CRH) and the tricyclic antidepressant desmethylinipramine (DMI) potentiate cocaine kindled seizure development

1. Project Description:

A. Objectives

Kindling refers to the progressive development of seizures following repeated, intermittent administration of a subconvulsant stimulus. Kindled seizures can be produced by direct electrical stimulation of the brain or by the administration of certain pharmacological agents. This project investigates the development and completed phases of local anesthetic kindling using cocaine and lidocaine. Pharmacological interventions to slow the rate of kindled seizure development are attempted and, as carbamazepine has been demonstrated to be a highly effective agent in the prevention of local anesthetic kindled seizures, this model is also used to attempt to elucidate carbamazepine's mechanism of action. Finally studies are being conducted to examine potential substrates of the local anesthetic kindling process using in situ hybridization of c-fos (Z01 MH 02460-02 BPB) and CRH mRNA (with Mark Smith and Phil Gold: CNE).

B. Methods Employed

1. Subjects: Male Sprague Dawley rats (200-300 g).

2. Procedures:

a. Pharmacological kindling is accomplished by once daily intraperitoneal (i.p.) administration of local anesthetics lidocaine (65-85 mg/kg) or cocaine (40-65 mg/kg). The animals are observed for seizures, death, stereotypy (for cocaine) and aggression (for lidocaine). A number of different pharmacological agents are administered (i.p. or in a diet preparation) before the lidocaine or cocaine in attempt to alter their effects.

b. Animals are kindled and sacrificed for in situ hybridization or other biochemical assays.

3. Findings:

1. Chronic carbamazepine markedly inhibits the development of cocaine and lidocaine kindled seizures and cocaine seizure-induced lethality. Acute administration of carbamazepine in doses of 15-50 mg/kg is without effect on local anesthetic seizures and repeated acute administration (daily, prior to each cocaine or lidocaine treatment) was either without effect (carba-15 mg/kg) or worsened the seizure development and lethality (carba-50 mg/kg). A number of control studies were conducted to rule out the possibility of different blood levels of carbamazepine or its epoxide metabolite following i.p. as compared to chronic oral treatment, and different pretreatment regimens. All of the data pointed to the importance of the chronic continuous carbamazepine treatment regimen.

2. The effect of a range of doses of corticotropin-releasing-hormone (CRH) (1, 5, 10, 100 ug, i.c.v.) on cocaine seizure development and lethality, and on carbamazepine's anticonvulsant effects was evaluated. Local anesthetics release CRH, in vitro and this effect was blocked by carbamazepine. Moreover, CRH itself, produces limbic seizures, and manipulation of CRH levels through feedback mechanisms (i.e. RU-486 or glucocorticoid administration) can alter the rate of development of local anesthetic seizures. CRH, at all doses tested, was found to markedly potentiate

cocaine kindling, and its associated lethality. A reversal of carbamazepine's anticonvulsant effects was seen only with the higher doses of CRH (10 and 100 ug) suggesting a lack of specificity for carbamazepine's anticonvulsant mechanisms of action. Parenthetically, all the animals that received the higher doses of CRH (10 and 100 ug) were also watched for CRH-induced seizures (for 3-8 hours after CRH administration). No effect of carbamazepine on the incidence or latency of CRH-induced seizures was observed. Since CRH is released during stress, and potentiates cocaine's toxic effects, another potential liability for cocaine is suggested by these data.

3. Chronic carbamazepine treatment up-regulates adenosine receptors in a long-lasting fashion (at least 2 months following carbamazepine discontinuation). Caffeine is an adenosine antagonist, and produces a similar up-regulation following chronic treatment. Functionally, an up-regulated adenosine receptor system could result in an enhanced response to endogenous adenosine, which has been implicated in seizure development processes. We examined the effect of chronic caffeine administration (600 mg caffeine/ kg diet) on cocaine kindled seizures and found a slowing of the kindling process in animals that had been pretreated with the caffeine diet (for 3 weeks), but were taken off of the diet when cocaine administration was begun. Those animals that remained on the caffeine diet throughout the experiment were not affected (compared to control-diet-treated animals). This finding suggests that an up-regulated adenosine system (without an antagonist concurrently administered) can slow the development of cocaine kindling. Thus, adenosine up-regulation remains a candidate mechanism for carbamazepine's anticonvulsant effects, however unlike caffeine, carbamazepine can be administered concurrently and produce its anticonvulsant effects. Differences between carbamazepine and caffeine's effects on adenosine have been investigated in our branch by Dr. Mike Clark.

4. The cholinergic agents atropine (20 mg/kg) and physostigmine (0.5 mg/kg) were investigated for their effects on cocaine and lidocaine kindling and on carbamazepine's anticonvulsant mechanism of action. Surprisingly, the effect of atropine was opposite for cocaine and lidocaine kindling. Atropine slowed cocaine kindling and its associated lethality, while it potentiated lidocaine kindling. Physostigmine had no effect on cocaine kindling and had a modest anticonvulsant effect on lidocaine seizure development. The effects of these agents on carbamazepine's anticonvulsant effects were consistent with their effects on the local anesthetic seizures, i.e., atropine potentiated carbamazepine's effects on cocaine kindling and reversed its effects on lidocaine kindling. No effect of physostigmine on carbamazepine's anticonvulsant effects was observed. These data suggest differential cholinergic modulation of cocaine compared to lidocaine kindling, the mechanisms of which remain to be determined.

5. Depletion of serotonin (using PCPA) did not affect carbamazepine's anticonvulsant efficacy, and only slightly potentiated cocaine kindling.

6. Depletion of somatostatin using cysteamine potentiated cocaine kindling and its associated lethality. This is in contrast to cysteamine's anticonvulsant effects on amygdala kindled seizures, and suggests that

carbamazepine's ability to lower somatostatin levels is unrelated to its anticonvulsant effect on local anesthetic kindled seizures.

7. The tricyclic antidepressant DMI also potentiated cocaine kindling and lethality, suggesting that the tricyclic structure of carbamazepine is not critical to its anticonvulsant effects, and also that caution should be used in the treatment of cocaine abuse with DMI.

8. Studies of the biochemical mechanisms of pharmacological kindling included evaluation of the in situ hybridization of c-fos m-RNA (see report of Dr. Mike Clark), and the effect of lidocaine kindling and carbamazepine on sodium channel mechanisms (with Drs. Russell Margolis and D.M. Chuang).

C. Proposed Course of Project

The studies above will continue with further attempts to dissect out carbamazepine's mechanisms of action in this paradigm. This seizure paradigm is unique in that it requires chronic, continuous treatment with carbamazepine and does not respond to acute drug administration, even at high doses. Thus, mechanisms engendered by chronic carbamazepine treatment may be elucidated in this paradigm, which may be relevant to carbamazepine's mechanisms of action in psychiatric illness.

Other studies will be directed at elucidation of the biochemical substrates of pharmacological kindling using mRNA probes for peptides such as CRH and dynorphin, and direct and indirect measures of sodium channel function.

D. Significance to Biomedical Research and the Program of the Institute

These studies may bring about mechanistic insights into the differences in adaptive mechanisms that occur with chronic vs. acute drug treatment. Moreover, the determination of carbamazepine's mechanism of action could be of use in the understanding of manic-depressive illness and in the development of other more specific or more effective treatment strategies. In addition, as a direct result of the findings regarding cocaine toxicity, carbamazepine is currently undergoing clinical trials as a potential treatment strategy for cocaine addiction. A patent has been issued to Drs. Post, Weiss and Thomas Aigner for this use of carbamazepine based on the preclinical findings.

Publications:

Post RM, Weiss SRB, Aigner TG. Carbamazepine in the treatment of cocaine abuse. In: Korenman SG, Barchas J, eds. Proceedings of the "conference on the biological basis of addiction". London, Oxford University Press, in press.

Post RM, Weiss SRB, Uhde TW, Clark M, Rosen JB. Preclinical neuroscience advances pertinent to panic disorder: implications of cocaine kindling, induction of the proto-oncogene c-fos, and contingent tolerance. In: Hoehn-Saric R, ed. Biology of anxiety disorders: recent developments. Washington DC, APA Press, in press.

Weiss SRB, Nierenberg J, Lewis R, Post RM. Corticotropin-releasing hormone: potentiation of cocaine-kindled seizures and lethality. *Epilepsia* 1992;33:248-54.

Weiss SRB, Post RM, Aigner TG. Potential utility of the anticonvulsant carbamazepine in the treatment of cocaine abuse disorders: clinical and mechanistic implications. In: Watson RR, ed. *Alcohol and drug abuse reviews*, vol III. New Jersey, Humana Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 MH 02529-0 3 BP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological and biochemical studies of amygdala kindling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Susan R.B. Weiss, Ph.D., Chief, Unit of Behavioral Biology, BPB, NIMH

Robert M. Post, M.D., Chief, BPB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychobiology

INSTITUTE AND LOCATION

National Institute of Mental Health

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to understand and modulate the course of development of amygdala kindled seizures. The effects of carbamazepine and other anticonvulsants on amygdala kindling have been examined in relation to stage of kindled seizure development (as well as type of kindling stimulus; see above--pharmacological kindling). Agents with specific biological target systems have been used to attempt to modulate carbamazepine's anticonvulsant effects on kindled seizures, in order to elucidate carbamazepine's mechanisms of action. Finally, studies addressing possible mechanisms of amygdala kindling have been conducted with Drs. Mike Clark, Jeff Rosen, Russel Margolis, and DeMaw Chuang (BPB), and Drs. Mark Smith and Phil Gold (CNE Branch). Significant findings to date include demonstration of the following: 1) carbamazepine is an effective anticonvulsant agent during the completed phase of amygdala kindling, but not during seizure development; 2) valproic acid is an effective anticonvulsant agent against both seizure development and completed seizures; 3) carbamazepine's anticonvulsant effects can be reversed by agents that act at the peripheral-type benzodiazepine receptor (Ro5-4864) and the alpha-2-noradrenergic receptor (yohimbine); 4) amygdala kindled seizures, electroconvulsive shock seizures, and afterdischarge activity in the amygdala (without generalized seizures) can induce CRH-mRNA in the hippocampus, in cells which do not normally express CRH message; 5) lesions of the olfactory bulb do not affect the development of amygdala kindled seizures or anticonvulsant responsivity to carbamazepine, valproate, or diazepam; 6) the absence of seizures in kindled rats produces anticonvulsant refractoriness upon subsequent testing; 7) the proto-oncogene c-fos is induced in a regionally selective manner during kindling development, which, in the early stages of kindling, is dependent upon the length of the elicited afterdischarge duration; and 8) the mRNA for TRH also increased with amygdala kindling, in roughly the same areas as the c-fos expression.

1. Project Description

a. Objectives

The project on amygdala kindling has two main purposes: 1) to evaluate the anticonvulsant effects of certain agents, with consideration given to different stages of seizure evolution; and 2) to elucidate biochemical substrates of amygdala kindling. Amygdala kindled seizures provide reliable and robust behavioral and electrophysiological endpoints, which facilitate the evaluation of drug effects. Moreover, carbamazepine is now one of a variety of anticonvulsant agents that are being evaluated for treatment of psychiatric illnesses. Additionally, electrical kindling is a good model for studying neuronal plasticity, as electrophysiological responsivity changes gradually over time and these changes, once induced, remain on a permanent basis.

b. Methods employed

1. Subjects: Male Sprague Dawley rats, 300-500 g.

2. Procedures:

a. Rats are surgically implanted with an electrode in the amygdala and given 1 second of electrical stimulation daily. Electroencephalographic recordings from the amygdala and seizure stage (or intensity) are evaluated. Drugs may be administered at various times during the kindling process to attempt to alter the development of kindled seizures, or to block the completed kindled seizures. Combinations of drugs may be given to try to block the anticonvulsant effects of a known effective agent.

b. Rats are kindled in the same fashion as described above, however no drugs are given and the animals are sacrificed at specific times in the kindling process to evaluate biochemical alterations.

c. Rats received lesions of the olfactory bulbs prior to kindling for evaluation of kindled seizure development and anticonvulsant drug effects.

3. Findings:

1. Carbamazepine, and its 10'll-epoxide metabolite are ineffective in preventing the development of amygdala kindled seizures, however, both drugs are highly effective on completed kindled seizures. This alteration in pharmacological responsivity, based on stage of the kindling process, has interesting clinical implications regarding different treatment strategies depending partly upon the course of evolution of the illness.

2. Carbamazepine's acute anticonvulsant effects on amygdala kindled seizures are blocked by the administration of Ro5-4864, an agonist at the peripheral-type benzodiazepine receptor, and the alpha-2-adrenergic antagonist yohimbine. These data suggest an involvement of these systems in carbamazepine's mechanism of anticonvulsant action.

3. Valproic acid is an effective agent in slowing the development of amygdala kindling, and against completed amygdala kindled seizures. The mechanisms of action of valproic acid are being investigated but the data on contingent tolerance and cross tolerance to carbamazepine (see report # Z01 MH

02530-01 BP) raise the possibility that the peripheral-type benzodiazepine receptor may be indirectly involved.

4. Valproic acid was reported to distribute almost exclusively in the olfactory bulbs of rats. Since the peripheral-type benzodiazepine receptor is also found most densely in this region in rat brain, the effect of olfactory bulbectomy was evaluated on kindled seizure development and anticonvulsant responsiveness. No effect was seen on kindling, or on the anticonvulsant effects of carbamazepine, valproate or diazepam. Thus, the peripheral-type benzodiazepine receptors in the olfactory bulb do not appear critical to the anticonvulsant efficacy of these compounds. Moreover, no effect of the lesion was observed on contingent tolerance development to carbamazepine (see report # Z01 MH 02530-01 BP).

5. The induction of c-fos mRNA during the amygdala kindled seizure process has been extensively studied by Mike Clark and is reported in detail in Z01 MH 02460-02 BP.

6. Using in situ hybridization techniques, in collaboration with Mark Smith and Phil Gold (CNE), we found an induction of CRH mRNA in the hippocampus of animals that had amygdala kindled seizures, afterdischarge activity without generalized seizures, and electroconvulsive shock seizures. Similar changes were not seen following stress or adrenalectomy, in which case alterations were seen in hypothalamic CRH mRNA. These data suggest differential regulation of CRH synthesis in hippocampus as compared to hypothalamus, with the latter being more clearly related to stress and glucocorticoid effects. The importance of kindling per se in the regulation of hippocampal CRH is not clear since similar effects were seen with ECS seizures (which do not kindle). Seizures, as well, were not critical to the effect in the hippocampus, since animals that only experienced focal epileptiform activity demonstrated the same changes in CRH mRNA.

7. The anticonvulsant effect of carbamazepine was evaluated in animals that had been kindled every day vs. those that a period of time off from seizure stimulation. Periods 4 days-2 week without seizures induced refractoriness to carbamazepine while 1-2 day of time off did not interfere with carbamazepine's efficacy. Potential biochemical mechanisms of this effect are being studied, using the time course for this effect as a means of distinguishing candidate processes.

8. The induction of TRH mRNA during the amygdala kindled seizure process has been extensively studied by Jeff Rosen and is reported in detail in Z01 MH 02524-02 BP.

C. Proposed Course of Project

Investigation into the mechanisms of action of valproate will be continued as this anticonvulsant, in particular, has been very promising in clinical trials for manic-depressive illness. Other investigations into biochemical mechanisms associated with kindling development will continue with specific emphasis on changes in second and third messenger systems (e.g., protein phosphorylation, immediate early gene and peptide RNA expression) and sodium and calcium channel function.

The finding that anticonvulsant refractoriness occurs with time-off from seizures implies that seizures are capable of inducing endogenous anticonvulsant alterations which last for at least 2 days (but not 4 days). We will first

determine the generality of this finding with regard to other anticonvulsant treatments, and subsequently investigate putative endogenous anticonvulsant mechanisms associated with seizure episodes (e.g., changes in neuropeptides, receptors, 2nd messenger systems...). Potential strategies for enhancing the beneficial effects of the seizures will also be investigated.

D. Significance to Biomedical Research and the Program of the Institute

These studies provide an important clinical insight into novel factors that should be considered with respect to pharmacological responsivity. They have also provided information regarding carbamazepine's mechanisms of action and the function of the peripheral-type benzodiazepine receptor, which has not been functionally well characterized up to this point. The mechanisms of kindling and neuronal plasticity are of great interest, and we have demonstrated a novel mechanism in which cells that do not normally express CRH mRNA do so in response to kindling stimulation or seizures. We have also just discovered an experimental leverage point for studying how pathological processes (seizures) can also be inducing endogenous compensatory therapeutic alterations.

Publications:

Post RM, Weiss SRB. Endogenous biochemical abnormalities in affective illness: therapeutic vs. pathogenic. (Ziskind-Somerfeld Research Award Paper) Biol Psychiatry 1992, in press.

Post RM, Weiss SRB, Clark M, Nakajima T, Ketter T. Seizures as an evolving process: implications for neuropsychiatric illness. In: Theodore WH, Devinsky O, eds. Epilepsy and behavior. New York, Alan R Liss Inc, 1991;361-87.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02530-03 BP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Contingent Inefficacy and Contingent Tolerance		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Susan R.B. Weiss, Ph.D., Chief, Unit of Behavioral Biology, BPB, NIMH Robert M. Post, Chief, BPB, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Psychobiology		
INSTITUTE AND LOCATION National Institute of Mental Health		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The overall objectives of this project are to the study the phenomenology and biological substrates of contingent drug effects. Using a kindling paradigm, we have demonstrated that the anticonvulsant efficacy of carbamazepine, and other drugs, could be manipulated by temporal factors relating to drug administration and seizure presentation. Significant findings to date include demonstration of the following: 1) contingent inefficacy, whereby the contingent presentation of carbamazepine during amygdala kindling seizure development (i.e., before, but not after electrical stimulation), while not affecting kindling development, produced a subsequent refractoriness to carbamazepine's anticonvulsant effects on completed kindled seizures (when it should have been effective); 2) contingent tolerance, in which animals that have completed kindled seizures develop tolerance to carbamazepine following repeated administration of the drug prior to, but not after, each electrical stimulation; 3) contingent tolerance reversal by treatment with carbamazepine after the kindled seizures or kindled seizures alone (no drug), but not by time off (no drug or kindling stimulation) for periods of up to three weeks; 4) contingent refractoriness to valproate, in which animals that were kindled with the contingent presentation of valproate before each stimulation (which slowed kindling development), became valproate non-responsive; those kindled with non-contingent exposure to valproate remained sensitive to its anticonvulsant effects; 5) cross tolerance to carbamazepine in valproate-refractory rats; and reversibility of this effect by kindling the animals with valproate after each stimulation for one week; 6) cross tolerance between carbamazepine and a ligand that binds the peripheral-type benzodiazepine receptor, and valproic acid, but not between carbamazepine and diazepam; 7) alterations in seizure threshold which mirror the changes in responsivity to carbamazepine; 8) slowing of contingent tolerance development by non-contingent drug presentation or by kindling the rats at lower stimulation currents, but not by higher doses of carbamazepine; 9) modulation of kindled seizure thresholds by different levels of kindling stimulation; 10) no effect of the NMDA antagonist MK-801 or the calcium channel antagonist nimodipine on contingent tolerance development.		

1. Project Description

A. Objectives

The project on contingent inefficacy and tolerance is to characterize these novel phenomena regarding anticonvulsant drug efficacy, and to attempt to elucidate underlying mechanisms. The importance of temporal contingencies of drug presentation, even for a response that is as physiologically hard-wired as a seizure, suggests the basic importance of such phenomena in a wide range of drug effects. In addition, since we have uncovered a robust and reliable paradigm for modulating seizure thresholds in kindled animals, elucidation of the variables controlling this is being attempted. If clinical pathological changes can be subject to similar modification, then these data may be generative of novel treatment strategies.

B. Methods Employed

1. Subjects: Male Sprague Dawley rats, 300-500 g.

2. Procedures:

a. Amygdala kindling: Rats are surgically implanted with an electrode in the amygdala and given 1 second of electrical stimulation daily. Electroencephalographic recordings from the amygdala and seizure stage (or intensity) are evaluated. Drugs are administered at various times during the kindling process to attempt to alter the development of kindled seizures, or to block the completed kindled seizures.

3. Findings:

a. The novel phenomena of contingent inefficacy in which treatment with carbamazepine during seizure development, before but not after the electrical stimulation, produces a subsequent refractoriness to carbamazepine's anticonvulsant effects on completed kindled seizures. The refractoriness could be partially reversed by treatment for a week with carbamazepine given after the stimulation.

b. Contingent tolerance to carbamazepine in animals with completed kindled seizures, in which the repeated administration of carbamazepine, before but not after stimulation, results in its loss of anticonvulsant efficacy. Tolerance could be reversed by kindling the animals without drug, or with carbamazepine given after the kindling stimulation for periods of 5 days or longer. Tolerance did not reverse by a period of time off from both stimulation and drug (11 days or three weeks), suggesting that a specific unpairing of the seizure and drug administration was required.

c. Contingent refractoriness to valproate in which animals that were kindled with valproate before each stimulation become resistant to valproate's anticonvulsant effects when they developed fully kindled seizures. Those rats receiving valproate after stimulation did not lose valproate efficacy, again ruling out pharmacokinetic variables in this effect. Rats that were not responsive to valproate also were unresponsive to carbamazepine, and a reinstatement of efficacy to both drugs was accomplished by one week of kindling the animals with valproate after each stimulation.

d. Contingent tolerance was also demonstrated in the lidocaine kindling paradigm using diazepam: tolerance could be reversed by a period of 5 days of lidocaine kindling with diazepam administered after the seizures.

e. Tolerance to carbamazepine could be delayed by the addition of non-contingent drug presentation, but not by increasing the dose of carbamazepine, or by daily alternating drug treatment with diazepam.

f. Cross tolerance was demonstrated to the peripheral benzodiazepine ligand PK-11195, further implicating this site in carbamazepine's anticonvulsant actions. No cross tolerance was seen to diazepam, which acts through the central benzodiazepine receptor system.

g. Cross tolerance was also seen between carbamazepine and valproate.

h. Generalized seizure thresholds were evaluated, in the non-drugged state, as a measure of seizure susceptibility in animals that were made tolerant to carbamazepine and then following a reinstatement of efficacy (tolerance reversal). Seizure thresholds decreased when the animals were tolerant and returned to kindled baseline when the animals were made responsive. This effect was replicated 3 times in the same group of animals and again in a separate group of kindled rats.

i. Based on the above findings, it was postulated that the rate of tolerance development could be manipulated by the level of stimulation current. If the stimulation current was high, then a small drop in threshold might be sufficient to override carbamazepine's threshold-increasing effects and induce tolerance. If the stimulation current was just above threshold, then a larger drop in threshold would be required for tolerance to develop. Stimulation at threshold current (plus $50\mu\text{A}$) produced slower tolerance development than stimulation at suprathreshold current ($800\mu\text{A}$). This effect was demonstrated in a double crossover design in which between group comparisons were significant as well as within group comparisons.

j. Based upon the above findings of compensatory proconvulsant responses (threshold decreases) developing in conjunction with contingent anticonvulsant treatment, it was considered that compensatory anticonvulsant responses could be generated by manipulation of the proconvulsant stimulation. This was evaluated by kindling two groups of animals at threshold (+ $50\mu\text{A}$) or suprathreshold levels of stimulation and then assessing responsiveness to different levels of stimulation current. Seizures developed in both groups of animals at approximately equal rates. However, when the animals that were kindled at suprathreshold levels were switched to their previously determined threshold (+ $50\mu\text{A}$), they no longer had seizures. The rats kindled at threshold, when given one week of exposure to suprathreshold stimulation, no longer responded to their previous threshold level of stimulation. This effect lasted for approximately one week and when the seizures returned they were full blown rather than developing through stages as occurs in kindling development. Thus, a permanent reversal of the kindling process did not occur, however, long lasting anticonvulsant effects were observed by kindling with a more intense stimulus. The implications of these findings are that greater anticonvulsant compensatory mechanisms are engendered by higher levels of electrical stimulation. Moreover, even kindled animals, which retain a permanent alteration in their susceptibility to seizures, have the possibility of potential clinical improvement through threshold manipulation.

k. Since NMDA receptors have been implicated in the acquisition of LTP and in other tolerance paradigms, we evaluated the effects of the NMDA

receptor antagonist MK-801 on contingent tolerance development to carbamazepine. No effect was observed. The calcium channel antagonist nimodipine has also been implicated in drug tolerance and memory paradigms, and was also without effect on contingent tolerance development to carbamazepine.

1. Potential substrates of contingent tolerance development are being evaluated using a number of different measures, e.g. protein kinase C translocation (with Russell Margolis and James Olds), GABA and benzodiazepine receptor function (with Mike Clark), TRH, enkephalin, dynorphin and somatostatin mRNA expression (with Jeff Rosen). The results of these studies are currently being analyzed.

C. Proposed Course of Project

Behavioral and pharmacological methods to slow down contingent tolerance development will be further investigated for their potential clinical importance. Examination of potential biochemical concomitants of this effect will continue to be undertaken, in collaboration with Drs. De-Maw Chuang, Jeff Rosen, Mike Clark and Mark Smith. Other studies will focus on potential mechanisms of the threshold changes that are induced.

D. Significance to Biomedical Research and the Program of the Institute

These phenomena illustrate rather dramatically a fairly unrecognized component of loss of drug efficacy. They further suggest that reinstatement of drug responsiveness may be accomplished through manipulation of temporal or other contingencies of drug administration. The data also point out the importance of non-pharmacokinetic factors in drug effectiveness. Moreover, this paradigm offers a novel entree into the study of learning mechanisms as the effect is robust, and requires only the manipulation of temporal contingencies or stimulation parameters.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02531-03 BP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Alterations in Brain Neurochemistry as Assessed with Microdialysis Procedures		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Agu Pert, Ph.D., Chief, Unit on Behavioral Pharmacology, BPB, NIMH Nigel Thomas, Ph.D., MacArthur Post-doctoral Fellow, BPB, NIMH Krystyna Wozniak, Ph.D., Visiting Fellow, LCS, NIAAA Markku Linnoila, M.D., Chief, LCS, NIAAA Lynda Erinoff, Ph.D., Program Officer, NRB, NIDA Margaret Hamilton, Ph.D., Fogarty Fellow, BPB, NIMH Alessandro Zocchi, Ph.D., Fogarty Fellow, BPB, NIMH Richard Rothman, M.D., Ph.D., ARC, NIDA		
COOPERATING UNITS (if any) LCS, NIAAA; NRB, ARC, NIDA;		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Behavioral Pharmacology		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland		
TOTAL STAFF YEARS: <div style="text-align: center;">1.8</div>	PROFESSIONAL: <div style="text-align: center;">1.6</div>	OTHER: <div style="text-align: center;">.2</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Direct applications of <u>cocaine</u> to the <u>striatum</u> via <u>microdialysis probes</u> produced a concentration-dependent <u>increase in dopamine overflow</u>, while applications to the <u>amygdala</u> were <u>ineffective</u>. <u>Amphetamine</u> was also considerably more potent in the <u>striatum</u>, suggesting differences in <u>DA transporters</u> in the <u>two brain regions</u>. <u>Cocaine</u> was found to increase <u>striatal ACh</u> in both anesthetized and unanesthetized rats. <u>Morphine</u>, at 10 mg/kg, also elevated striatal ACh. <u>MK-801</u> at low doses (0.1 mg/kg), on the other hand, produced a <u>modest decrease in extracellular levels of the transmitter</u>. Applications of cocaine to the <u>hippocampus</u> produced a <u>concentration-dependent increase in extracellular NE</u>. In the frontal cortex, on the other hand, only the highest concentration (100 µm) produces an increase in extracellular NE. Systemic administration of cocaine (20 mg/kg i.p.) produced no effect on extracellular NE in either the <u>hippocampus</u> or <u>frontal cortex</u>. The differential effects of cocaine in the two regions may suggest differences in the affinity of cocaine for the NE uptake sites. <u>Fluoxetine</u> applied focally to the frontal cortex and raphe increased extracellular 5-HT. A concurrent decrease of 20% in 5-HT occurred in each normally perfused region following focally applied fluoxetine at the other site. <u>Systemic fluoxetine increased 5-HT</u> in the raphe but decreased it in the frontal cortex. The net effect of systemic fluoxetine appears to be determined predominantly by increased 5-HT in the somatodendritic region, which inhibits raphe firing, resulting in a decrease in cortical release. <u>Electrical stimulation</u> of the VTA as well as injections of <u>enkephalin</u> into this region produced increases in the <u>nucleus accumbens DA</u> and <u>locomotor activity</u>. These two effects appeared to be dissociated. </p>		

Other professional personnel (continued):

David Fontana, Ph.D., MacArthur Fellow, BPB, NIMH
Robert M. Post, M.D., Chief, BPB, NIMH

I. Project Description

A. Objectives

A variety of psychoactive compounds have reinforcing properties in animals and produce euphoria in man. These actions are thought to be mediated through activation of mesolimbic dopaminergic mechanisms, although the participation of other neurotransmitters has also been suggested. The purpose of this project was to examine the interaction of various pharmacological agents with the brain amine as well as acetylcholine systems.

B. Methods Employed

1. Effects of cocaine and amphetamine overflow in the amygdala and striatum

There are reasons to suspect that dopamine (DA) transporters are not the same in all regions of rat brain. The purpose of these studies was to compare the actions of two compounds that depend on transporter function on DA overflow in two different brain regions. A microdialysis probe was introduced into the striatum or amygdala of anesthetized rats. These structures were perfused with artificial CSF containing varying amounts of either amphetamine or cocaine. Cocaine was also administered intravenously to animals with probes in the amygdala or striatum.

2. Effects of cocaine, morphine, and MK-801 on striatal acetylcholine overflow

Cocaine, morphine, and MK-801 produce increases in locomotor behaviors in rats through different initial receptor interactions. One common link through which these drugs may exert at least part of their behavioral effects is the cholinergic interneuron in the striatum. The purpose of this study was to evaluate the effects of these three compounds on striatal acetylcholine (ACh) following systemic injections. The effects of cocaine on ACh were examined in both anesthetized and awake preparations. For the latter, rats were implanted with CMA guide cannulae aimed for the striatum. One week following surgery, microdialysis probes were introduced into the striatum and perfused with artificial CSF containing 10 μ M neostigmine at 0.5 μ l/min. The animals in the anesthetized study were injected with chloral hydrate, placed in a stereotaxic frame, and then had dialysis probes introduced into the striatum. Dialysates were analyzed for ACh and choline by a reverse phase HPLC system with electrochemical detection.

3. Regional changes in extracellular norepinephrine following focal and systemic cocaine

Cocaine prevents the neuronal reuptake of brain catecholamines and indoleamines. Alterations in DA function have been studied extensively and presumably underlie the majority of the behavioral effects of this psychomotor stimulant. However, considerably less is known about the actions of cocaine on the norepinephrine (NE) system. In the present study using *in vivo* microdialysis, we have characterized the effects of focal and systemic cocaine on extracellular NE in two noradrenergic terminal regions, the hippocampus (HI) and frontal cortex (FC). Male Sprague-Dawley rats were anesthetized with chloral hydrate and dialysis probes were stereotaxically implanted into either the HI or FC. Basal NE concentrations in the HI and FC were 5.8 ± 0.21 pg/sample (mean \pm SEM, n=4) and

4.2 ± 0.3 pg/sample ($n=4$), respectively. Following stabilization of basal NE, cocaine was applied focally via the dialysis probe at concentrations of 1, 10 and $100 \mu\text{M}$ for 15 min. Sampling was then continued for a further four samples prior to application of the successive concentration of cocaine.

4. Alterations in raphe and frontal cortex serotonin overflow following focal and systemic administration of fluoxetine

Fluoxetine is an antidepressant drug that is a potent inhibitor of 5-hydroxytryptamine (5-HT) reuptake. This study describes *in vivo* assessment of this compound on serotonergic transmission in two brain regions. Male Sprague-Dawley rats were anesthetized with chloral hydrate and stereotactically implanted with concentric dialysis probes into frontal cortex and raphe nuclei. Microdialysis samples were collected and assayed for 5-HT content. After basal levels of 5-HT were attained in both areas, fluoxetine was applied either focally into one region or administered systemically.

5. Alteration in mesolimbic dopamine overflow by opioids and electrical brain stimulation

Considerable electrophysiological, pharmacological, and behavioral evidence suggests an important interaction between endogenous opioids and mesolimbic DA neurons. The present study focused on comparing the ability of two different routes of opioid administration, either systemic or microinjection into the ventral tegmental area (VTA), with electrical stimulation of DA cell bodies in the VTA to: 1) influence extracellular levels of DA in the n. accumbens, and, 2) elicit locomotor activity. Animals were implanted with guide cannulae aimed for the n. accumbens. Following recovery from surgery, microdialysis probes were introduced into the n. accumbens through the guides. Dialysates were assayed for DA and DA metabolites as before. DA overflow was assessed following either systemic injections of morphine, VTA injections of enkephalin, or electrical stimulation of the VTA in the presence and absence of nomifensine.

C. Major Findings

Whereas the application of cocaine (0.01, 0.1, and 1 mM) into the striatum increased DA overflow in a concentration-related manner, it produced either weak or inconsistent effects in the amygdala. (A difference in sensitivity between the striatum and amygdala was also found, albeit much less marked, when cocaine was administered systemically (40 mg/kg, i.p.). In the case of amphetamine (0.1, 1, and $10 \mu\text{M}$), its application into the striatum also increased DA overflow in a concentration-related manner; unlike cocaine, however, amphetamine produced consistent concentration-related increases when administered into the amygdala (even though the striatum was apparently more sensitive). These findings suggest that the effects of amphetamine, and especially cocaine, differ depending on whether the drugs are administered into the striatum or amygdala. The possibility is raised, then, that cocaine binding sites or DA transporters located in the striatum differ from those located in the amygdala.

Cocaine (20 mg/kg i.p.) increased striatal ACh levels approximately 50% during the first three 20-min sampling periods in unanesthetized rats. The increase in ACh was even more pronounced in anesthetized rats (peak effect of approximately 160% increase during the second sampling period). Systemic

administration of MK-801 (0.5 mg/kg) had little if any effect on striatal ACh in anesthetized rats. In freely moving awake rats, 0.25 μ g/kg of MK-801 also had little effect while 0.1 ng/kg seemed to produce a slight decrease. Morphine at 10 ng/kg produced a significant elevation in striatal ACh while 20 ng/kg had a smaller effect.

In the HI, cocaine (1-100 μ M) produced a concentration-dependent increase in the extracellular NE of 20%, 52%, and 142%, respectively. However, in the FC, only the highest concentration of cocaine (100 μ M) produced an increase in the extracellular NE (63%). Systemic administration of cocaine (20 mg/kg i.p.) produced no effect on the extracellular NE in either the HI or the FC. These data are consistent with a previous report in which we have shown that desmethylinipramine administered focally into the two regions produces increases in extracellular NE, yet systemic administration is unable to alter extracellular NE in either region. The differential effects of cocaine in the two regions may suggest differences in the affinity of cocaine for the NE uptake site.

Focal fluoxetine (100 μ M) significantly increased local extracellular levels of 5-HT by approximately 400%. Both the frontal cortex and the raphe nuclei displayed similar sensitivity to fluoxetine perfusion. A concurrent decrease of 20% in 5-HT occurred in each normally perfused region following focally applied fluoxetine at the other site. Systemic fluoxetine (15 mg/kg i.p.) also significantly increased 5-HT by about 300% in the raphe nuclei, but in contrast there was a concurrent decrease in 5-HT (50%) in frontal cortex. This is an in vivo demonstration of opposite effects of systemic fluoxetine in two brain regions. Since reuptake blockade has similar effects on 5-HT in terminal as well as somatodendritic regions, it appears that the uptake sites have similar characteristics in both areas. A decrease in 5-HT overflow in the frontal cortex following application of fluoxetine to the raphe nuclei presumably reflects the activation of somatodendritic autoreceptors. The elevation of cortical 5-HT by focally applied fluoxetine on the other hand, probably activates feedback inhibitory pathways. The net effect of systemic fluoxetine appears to be determined predominantly by increased 5-HT in the somatodendritic regions which dramatically inhibits raphe neuron firing, resulting in a decrease in cortical release.

Mean increases in n. accumbens extracellular DA by the three treatments -- systemic or intracranial opioid administration or electrical stimulation of the DA cell body region -- remained modest regardless of treatment. The modest DA increases were coupled with elevated metabolite levels that persisted over time. Most DA metabolism is believed to involve intraneuronal breakdown of recaptured DA. The effects on extracellular DA of the combined nomifensine/morphine or stimulation treatments appeared to be additive at best, suggesting that increases in the firing of DA neurons were not accompanied by an enhanced efficiency of the reuptake system that may have masked a greater effect of the treatments on DA release. Locomotor activity was most pronounced during electrical stimulation of the VTA, although increases in extracellular DA levels in the n. accumbens were not essentially different from those following either route of opioid administration. Systemic administration of morphine recruits all opioid receptor populations, including those involved in sedation. This would account for the lower levels of locomotor behavior in morphine-treated rats, including the

transient reduction in locomotor activity among rats pretreated with nomifensine. Intra-VTA administration of DALA presumably affected a discrete population of opioid receptors, and no sedation was observed. Locomotor behavior in this condition, although greater than that among morphine-treated rats, did not approach that occurring during electrical stimulation of the VTA. Opioids in the VTA exert a net excitatory influence on VTA-DA neurons projecting to the n. accumbens. This finding illustrates that a sparsely distributed receptor population such as that represented by opioid receptors in the VTA may exert a significant influence on other neuronal systems and on behavior. Opioids in the VTA may also participate in increasing DA synthesis as reflected by disproportionately elevated metabolite levels following intra-VTA DALA injections. Direct excitation of VTA-DA neurons by electrical stimulation produces a small increase in n. accumbens DA and a marked effect on locomotor activity. Coupled with the neurochemical and behavioral observations following opioid administration, this suggests that only a small increase in n. accumbens DA neurotransmission may be required to maximize behavioral expression. Differences in locomotor activity, but not DA overflow, following direct manipulation of VTA systems by electrical stimulation or opioid microinjection suggest that non-DA systems originating in the VTA may also participate importantly in locomotor behavior.

II. Significance to Biomedical Research and the Program of the Institute

A number of psychoactive drugs produce euphoria and act as reinforcers. It is thought that the reinforcing properties of these compounds are related to their ability to activate pleasure circuitry in the brain. Understanding the neural substrates underlying euphoria or pleasure could help to elucidate the neurobiological variables involved in affective disorders. Fluoxetine is a widely used and effective antidepressant. Understanding its mechanisms of action would help to elucidate the etiology of depression as well as aid in the development of more effective pharmacotherapeutics.

III. Proposed Course of Project

A. Further efforts will be made to define the regional effects of uptake blockers on norepinephrine overflow.

B. The regional effects of fluoxetine in 5-HT overflow will be characterized.

C. Alterations in 5-HT uptake and release will be evaluated following chronic fluoxetine.

D. Microdialysis as well as binding studies will be continued in order to characterize DA transporters in the frontal cortex, striatum, amygdala, and n. accumbens.

E. The mechanisms of action of cocaine on ACh overflow will be elucidated.

F. The effects of D₁ and D₂ selective agonists and antagonists on ACh overflow will be determined.

G. Attempts will be made to define the actions of nicotine on mesolimbic and nigrostriatal DA function.

H. Techniques will be established to measure GABA with microdialysis procedures.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02532-03 BP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Conditioned Determinants of Cocaine-Induced Behavioral Sensitization		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Agu Pert, Ph.D., Chief, Unit on Behavioral Pharmacology, BPB, NIMH Robert M. Post, M.D., Chief, BPB, NIMH Nigel Thomas, Ph.D., MacArthur Fellow, BPB, NIMH Richard Rothman, M.D., Ph.D., ARC, NIDA		
COOPERATING UNITS (if any) ARC, NIDA; MacArthur Foundation		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Behavioral Pharmacology		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland		
TOTAL STAFF YEARS: 1.4	PROFESSIONAL: 1.0	OTHER: .4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p> <u>Conditioned</u> effects of <u>cocaine</u> are relatively <u>resistant to extinction</u>. Following extinction, conditioned responses can be <u>reinstated</u> with a small <u>priming dose</u> of <u>cocaine</u>. The <u>magnitude</u> of conditioned increases in <u>locomotor activity</u> and <u>stereotypy</u> are dependent on the <u>conditioning dose</u> of cocaine. Injections of <u>cocaine</u> into the <u>n. accumbens</u> are also able to elicit <u>conditioned effects</u>. Unlike cocaine, <u>amphetamine</u> does not appear to support <u>conditioning</u> following one training session. A <u>blockade of glutamate</u> function with MK-801, a noncompetitive <u>antagonist</u>, prevents <u>cocaine-induced conditioning</u>. ECS delivered immediately following a <u>cocaine conditioning</u> session <u>prevents cocaine-induced conditioning</u>. ECS delivered <u>one hour prior</u> and <u>one hour following</u> conditioning or <u>one hour prior to testing</u> was <u>ineffective</u>, illustrating that <u>cocaine-induced conditioning</u> is determined by <u>associative processes</u>. <u>Dopamine depleting lesions</u> of the <u>n. accumbens</u> and <u>amygdala</u> <u>prevent cocaine-induced conditioning</u>. <u>Dopamine depleting lesions</u> of the <u>frontal cortex</u> and <u>striatum</u> were not effective in preventing the <u>conditioned</u> locomotor effects of cocaine. <u>Lesions</u> of the <u>raphe</u> and <u>locus coeruleus</u> were equally <u>ineffective</u>. <u>Stimuli associated</u> with <u>cocaine</u> develop the ability to <u>release mesolimbic dopamine</u>. </p>		

I. Project Description

A. Objectives

It has become apparent that conditioning of drug effects to cues associated with cocaine plays a very critical role in the development and maintenance of cocaine-induced sensitization as well as the addictive process. For example, cues associated with cocaine administration appear to acquire the ability to enhance the effects of cocaine on subsequent presentations. The purpose of these studies was to further define the neuropharmacological and neuroanatomical substrates of these conditioned effects and to characterize the behavioral variables involved in their acquisition, retention, and extinction.

B. Methods Employed

We have previously demonstrated that the role of conditioning can be evaluated with a very simple and efficient paradigm. Three groups of rats are employed. On day one, the first group (PAIRED) is injected with a high dose of cocaine and placed in locomotor activity chambers. One hour following removal from the chambers, this group is injected with saline in the home cage. The second group (UNPAIRED) is injected with saline in the test chamber and a high dose of cocaine in the home cage. The third group (CONTROL) receives saline in both environments. On day two all animals are injected with a low dose of cocaine and placed in the activity chambers. The presence of conditioning is evidenced by the high activity scores of the PAIRED group on day two.

We have made further attempts to elucidate some of the neuropharmacological and behavioral factors involved in the conditioned component of sensitization using the one-day design outlined above in addition to more prolonged conditioning with similar treatment regimens.

1. Extinction of cocaine-induced conditioning

Little is known regarding the extinction of conditioned drug effects once established. In the following studies, we employed the same three groups outlined above (PAIRED, UNPAIRED and CONTROL) but extended the training for seven consecutive days. During the next seven days, all rats were injected with saline prior to placement in the locomotor chambers (Extinction). On day 15, all animals were injected with 10 mg/kg cocaine prior to placement in the locomotor chambers in order to determine whether it was possible to reinstate the conditioned response in extinguished animals.

2. Magnitude of the unconditioned stimulus (cocaine) as a determinant of conditioning

PAIRED and UNPAIRED rats were injected with either 5, 10, 20, 30, or 40 mg/kg of cocaine on day 1. On day 2, all of these animals were challenged with 10 mg/kg of cocaine prior to placement in the activity chamber. Two groups of control rats were also included. These animals received saline in both environments on day 1. One of these groups was injected with 10 mg/kg of cocaine on day 2 while the other received saline prior to testing.

3. Ability of centrally administered cocaine to elicit conditioned responses

Rats were implanted with bilateral stainless-steel cannulae guides aimed for an area 2 mm dorsal to the n. accumbens. Following recovery from surgery, they were divided into four groups. The first group was treated in the same manner as the PAIRED group described above, while the second group was treated similar to the UNPAIRED animals. The third and fourth groups received saline in both environments. On day 2, the first three groups were injected bilaterally with 10 µg of cocaine HCl in the n. accumbens prior to testing, while the fourth group was injected bilaterally with saline. One week later, the experiment was repeated with three days of conditioning.

4. Ability of amphetamine to produce conditioning in the one-day paradigm

Two groups of PAIRED rats and two groups of UNPAIRED rats were injected with 2.5 ng/kg of amphetamine sulfate prior to placement in the test apparatus while the UNPAIRED animals received a similar dose two hours following return to the home cage. One of the PAIRED and UNPAIRED groups was exposed to the test chamber for 30 min following drug injections, while the other two groups received a two hour exposure. On day 2, all animals were injected with 0.5 ng/kg amphetamine and then placed in the locomotor chambers for 30 min.

5. Effect of glutamate blockade on cocaine-induced conditioning

Four PAIRED and four UNPAIRED groups were used. Only the PAIRED group was pretreated with saline prior to the cocaine injection (40 mg/kg) while the other three received 0.25, 0.50, or 1.0 mg/kg of MK-801 30 min prior to cocaine. The UNPAIRED rats received similar drug regimens in the home cage. On day two, all animals were injected with 10 mg/kg of cocaine prior to placement in the test apparatus.

6. Temporal effects of ECS on cocaine-induced conditioning

We have previously reported that ECS delivered immediately following conditioning prevented the expression of the conditioned response on day 2. In the following studies, ECS (80mA AC for 3.5 sec) was delivered to PAIRED and UNPAIRED rats one hour prior to conditioning, one hour after conditioning, and one hour prior to the test session on day 2.

7. The role of specific brain amine systems in cocaine-induced conditioning

Bilateral lesions were made in the striatum, frontal cortex, and locus coeruleus with 6-OHDA. Rats lesioned in the striatum and frontal cortex were pretreated with desmethylinipramine to protect noradrenergic neurons, while rats lesioned in the locus coeruleus were pretreated with GBR-12909 to protect dopaminergic neurons. The mesencephalic raphe nuclei were lesioned with 5'7 DHT by injections into the dorsal and median raphe. Sham lesions were made by introducing injection cannulae to an area 2 mm dorsal to the nucleus in question.

Four groups of rats were used to assess the effects of each lesion. One of the lesioned groups and one of the sham groups were treated as the PAIRED rats above, while the other two groups were treated like the UNPAIRED animals. On day 2, all rats were challenged with 10 mg/kg of cocaine prior to testing.

8. Release of mesolimbic DA by stimuli associated with cocaine

There is strong evidence to suggest that the conditioned effects of cocaine may be mediated in part by increased release of dopamine. In this study, rats were implanted with guide cannulae aimed for the n. accumbens. One week following surgery, the animals were divided into three groups and treated as the PAIRED, UNPAIRED, or CONTROL rats described above. The next day, microdialysis probes were introduced into the n. accumbens and all animals were injected with a low dose of cocaine (10 mg/kg) prior to placement in the activity monitor. Dialysis samples were collected every 10 min and analyzed for DA and DA metabolites.

C. Major Findings

Rats conditioned seven days with cocaine were surprisingly resistant to extinction in comparison with the control groups. Conditioned effects were still seen during the fourth extinction session. No differences among the locomotor output of the three groups were found during the seventh extinction session. When rats were challenged with 10 mg/kg of cocaine after the last extinction session, however, the PAIRED group showed significantly greater locomotor output relative to the two control groups, indicating that it was possible to reinstate the conditioned response in extinguished animals by priming with a small dose of cocaine.

All doses of cocaine, with the exception of 5 mg/kg, were able to produce conditioned increases in locomotor activity. Neither 5 mg/kg nor 10 mg/kg produced conditioned increases in stereotypy. These data indicate that even relatively low doses of cocaine are capable of producing conditioning after only one exposure.

Injections of cocaine into the n. accumbens were effective in eliciting a conditioned response in the PAIRED rats after one day of conditioning. A similar conditioned response was found following three days of conditioning. These data indicate that the central actions of cocaine either elicit or accentuate the conditioned response on day 2.

Amphetamine proved to be relatively ineffective in producing conditioned increases in locomotor activity with the one day conditioning design despite the fact that it produced profound increases in locomotor activity on day 1. The reasons for this are not entirely apparent but may be related to the relatively weaker motivational properties of this psychomotor stimulant in comparison with cocaine.

Administration of MK-801 concurrently with cocaine on day 1 was effective in preventing cocaine-induced conditioning. Interestingly, blockade was seen even with the lowest dose (0.25 mg/kg) which actually enhanced cocaine-induced increases in locomotor activity on day 1.

ECS administered only immediately following the conditioning session was able to prevent cocaine-induced conditioning. ECS administered either one hour prior to training or one hour prior to testing had no effect, even though it depressed activity in both the PAIRED and UNPAIRED groups relative to the sham ECS animals.

These data indicate that cocaine-induced conditioning is determined by an associative process, the consolidation of which is disrupted by ECS.

We have previously found that dopaminergic mechanisms in the n. accumbens and amygdala are critically involved in mediating the conditioned effects of cocaine. Dopaminergic lesions of the frontal cortex and striatum were ineffective in altering the conditioned increases in locomotor activity, although the striatal lesions did appear to attenuate conditioned stereotypy. Noradrenergic lesions of the locus coeruleus and serotonergic lesions of the dorsal and median raphe were also ineffective in altering the conditioned increases in locomotor output, although the raphe lesions did seem to alter conditioned stereotypy.

Rats which had apparatus cues associated with cocaine on day 1 showed significantly greater increases in mesolimbic DA overflow as well as locomotor activity than the control groups.

II. Significance to Biomedical Research and the Program of the Institute

Some of the behavioral actions of psychomotor stimulants increase in intensity and duration with repetitive administration (behavioral sensitization). It has been suggested that this sensitization may be a useful model for understanding the longitudinal evolution of manic syndromes in man, including the increase in vulnerability to recurrence following successive episodes. Interestingly, we have found recently that one factor involved in such behavioral sensitization is heavily dependent on conditioning. This probably accounts for the longevity of sensitization once established. The goal of this project is to analyze the neural mechanisms underlying the conditioned aspects of cocaine-induced sensitization.

III. Proposed Course of Project

A. Parametric studies will continue in an effort to ascertain the precise variables that control conditioned sensitization to psychomotor stimulants.

B. Microdialysis procedures will be used to evaluate changes in dopamine function in the amygdala and striatum during the expression of cocaine-conditioned effects.

C. Microdialysis procedures will be used to follow changes in acetylcholine and GABA release during cocaine-induced conditioning.

D. Lesion studies will be completed to define the precise neuroanatomical substrates involved in cocaine-induced conditioning.

E. New operant procedures will be established to measure the incentive-motivational properties of stimuli associated with cocaine.

Publications:

Fontana DJ, Post RM, Pert A. Conditioned increases in mesolimbic dopamine overflow by stimuli associated with cocaine. Brain Res, in press.

Rothman RB, Pert A. Electroconvulsive shock prevents cocaine-induced conditioning. In: NIDA research monograph, problems of drug dependence, in press.

Thomas DN, Fontana DJ, Post RM, Weiss SRB, Pert A. The involvement of the mesocorticolimbic dopamine system in the conditioned effects of cocaine. In: NIDA research monograph, problems of drug dependence, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02534-03 BP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurobiological Mechanisms Underlying Cocaine-Induced Sensitization		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Agu Pert, Ph.D., Chief, Unit on Behavioral Pharmacology, BPB, NIMH David Fontana, Ph.D., MacArthur Fellow, BPB, NIMH Krystyna Wozniak, Ph.D., Visiting Associate, LCS, NIAAA		
COOPERATING UNITS (if any) LCS, NIAAA		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Behavioral Pharmacology		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland		
TOTAL STAFF YEARS: 1.0	PROFESSIONAL: .8	OTHER: .2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Attempts were made to elucidate the <u>neurochemical mechanisms</u> underlying <u>behavioral sensitization</u> induced by <u>chronic</u> administration of <u>amphetamine</u> . Rats were treated daily for seven days with 3 mg/kg of amphetamine and then challenged on day eight or eleven with a lower dose of this stimulant. <u>Microdialysis studies</u> , as well as <u>behavioral analyses</u> , were conducted on the challenge days. Amphetamine applied to the <u>terminal fields</u> of the <u>dopamine pathways</u> elicited a dose-dependent increase in <u>dopamine overflow</u> . No differences were found between animals tested <u>chronically</u> with <u>amphetamine</u> or <u>saline</u> tested either one or four days following termination of treatment. A <u>systemic challenge</u> (subcutaneous) at 1.0 mg/kg also did not reveal a difference in the dopamine response between the two groups. A challenge with <u>0.25 mg/kg</u> , however, one day following termination of treatment, did reveal a more prolonged <u>increase</u> in <u>striatal dopamine overflow</u> in the <u>chronic amphetamine</u> group, although the <u>peak effect</u> was no different from the controls. <u>Behavioral sensitization</u> was seen in both stereotypy and locomotor activity with the drug regimen used in these studies. While it is possible that <u>increased responsivity</u> of the <u>dopamine system</u> to <u>amphetamine</u> may underlie some aspects of <u>behavioral sensitization</u> , <u>pharmacokinetic factors</u> must also be considered.		

I. Project Description

A. Objectives

It is well known that repeated administration of amphetamine to experimental animals results in an increased abnormal behavioral response to a challenge dose of the drug. The precise mechanisms underlying this phenomenon of behavioral sensitization is unknown, although an enhanced synaptic accumulation of dopamine has been implicated. For example, a recent study using the technique of microdialysis has demonstrated that this hyperbehavioral response to amphetamine following an aggressive chronic treatment regimen, is accompanied by significantly elevated dopamine release in the ventral striatum. We have examined and compared the effects of various doses of amphetamine, applied both to the terminal regions and systemically on dopamine (DA) overflow in rats chronically treated with a more moderate amphetamine treatment regimen as compared with those receiving saline. Additionally, we have investigated some behavioral aspects of the response to systemic challenge doses of amphetamine following a similar treatment regimen to that studied in the dialysis experiments.

B. Methods Employed

Male Sprague-Dawley rats were anesthetized with chloral hydrate and placed in a stereotaxic frame. Concentric microdialysis probes were positioned into either the striatum or n. accumbens. Dialysate was collected every 20 min and assayed for DA and DA metabolites with standard HPLC procedures.

The animals were injected daily with 3 mg/kg of amphetamine or saline for 7 days. Subsequent experimental challenges were performed at 24 or 86 hours after the last injection. Both systemic and focal amphetamine challenges were employed. In the focal challenge experiments, various concentrations of amphetamine (0.0001 mM to 0.1 mM) in artificial CSF were administered for one 20 min period of perfusion or, in some experiments, for 3 X 20 min perfusion periods. In the systemic challenge experiments, amphetamine (0.25 at 1 mg/kg) were administered s.c. Samples were collected for 60-180 min after injection. In the behavioral studies, rats were injected i.p. with 3 mg/kg of amphetamine once daily for seven days. On day 8 (one day following termination of treatment), half the rats were placed in locomotor activity chambers for 30 min and injected with 1 mg/kg of amphetamine. The other half were treated in a similar fashion four days following termination of treatment. Locomotor activity was recorded in 10 min intervals for one hour following the amphetamine challenge. Stereotypy was rated 20 and 30 min following injection.

C. Major Findings

Amphetamine applied to the terminal region of the corpus striatum elicited dose-related increases in DA overflow. Chronic amphetamine-treated animals did not exhibit any differences in dopamine overflow following focal amphetamine challenge when compared with saline-treated controls. This was true for both the low (0.0001 mM) and high (0.01 mM) dose challenges of amphetamine. Similarly, there was no apparent difference between the amphetamine and saline pretreatment groups in response to challenge doses of amphetamine administered at either 1 or 4 days following termination of treatment.

Systemic injections of amphetamine subcutaneously elicited dose-related increases in dopamine overflow in anesthetized rats. The n. accumbens and corpus striatum displayed different sensitivities with regard to their peak dopamine effects, with the striatum being significantly more sensitive than the accumbens. Chronic amphetamine-treated animals showed no apparent difference in peak dopamine response when compared with saline animals following either the 0.25 or 1 mg/kg challenge dose of amphetamine. In contrast, there was a significant difference in the overall response of the amphetamine pretreated animals compared with saline following the 0.25 mg/kg challenge dose. However, this was not apparent in the response to the 1 mg/kg challenge.

Animals treated with the relatively moderate regimen in this study showed behavioral sensitization to both the locomotor and stereotypic effects of a 1 mg/kg amphetamine challenge. The data from the microdialysis study suggest that a sensitization of dopaminergic systems may be at least partially responsible for this, as has been suggested by other investigators. However, our results also suggest a possible pharmacokinetic component to the apparent sensitization phenomena associated with amphetamine.

II. Significance to Biomedical Research and the Program of the Institute

Chronic administration of psychomotor stimulants in man ultimately produces a constellation of behavioral alterations resembling psychosis. Chronic administration of cocaine and amphetamine to animals has, in fact, been used as an experimental model for psychosis. Elucidating the neurochemical mechanisms underlying the behavioral changes induced by repetitive exposure to these agents may be useful for understanding the etiology of some forms of mental disorders.

III. Proposed Course of Project

A. Studies using microdialysis procedures will be initiated to determine the role of GABA and glutamate in mediating the actions of chronic amphetamine and cocaine.

B. Alterations in acetylcholine function will be evaluated following acute and chronic cocaine.

C. The mechanisms underlying the actions of cocaine on acetylcholine function will be elucidated.

Publications:

Wozniak KM, Linnoila M, Pert A. Behavioral sensitization following chronic amphetamine treatment and associated alterations in dopamine. In: NIDA research monograph, problems of drug dependence, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02535-03 BP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurobiology of Non-competitive NMDA Antagonists		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Agu Pert, Ph.D., Chief, Unit on Behavioral Pharmacology, BPB, NIMH Alessandro Zocchi, Ph.D., Fogarty Fellow, BPB, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Behavioral Pharmacology		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland		
TOTAL STAFF YEARS: 1.8	PROFESSIONAL: 1.6	OTHER: .2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) Injections of the <u>GABA</u> agonist <u>muscimol</u> into the <u>globus pallidus</u> contralateral to a <u>6-OHDA medial forebrain bundle lesion</u> inhibited <u>ipsilateral rotational behavior</u> in rats injected with both <u>amphetamine</u> and <u>MK-801</u> . These findings suggest that both <u>stimulants</u> are exerting their <u>motoric effects</u> by <u>inhibiting GABA transmission</u> in the <u>globus pallidus</u> . Injections of <u>low doses of MK-801</u> to rats decreased <u>acetylcholine levels</u> in the <u>striatum</u> of freely moving rats, at the same time increasing <u>locomotor output</u> . These findings support the possibility that <u>acetylcholine interneurons</u> in the <u>striatum</u> may be under <u>glutamatergic regulation</u> .		

I. Project Description

A. Objectives

Phencyclidine (PCP) is a powerful psychotomimetic substance that produces psychopathological effects that mimic the primary symptoms of schizophrenia. Such actions are mediated through either blockade of excitatory amino acid functions or through blockade of dopamine reuptake. Many of the effects of PCP do appear to involve dopaminergic mechanisms. For example, low doses of PCP induce psychomotor stimulation and rotational behavior in rats lesioned unilaterally in the nigro-striatal pathway. In addition, PCP and other PCP-like compounds have been reported to alter dopamine (DA) and DA metabolites in brain. MK-801, on the other hand, has no effect on DA reuptake but produces a behavioral profile similar to PCP. The purpose of these studies was to further define the actions of noncompetitive glutamate blockers like MK-801 and PCP on basal ganglia functions.

B. Methods Employed

One possibility is that the motoric effects seen after amphetamine and MK-801 are expressed through a common final neural substrate although their initial pharmacological actions are different. Both amphetamine and MK-801 produce rotational behavior ipsilateral to a unilateral lesion of the nigrostriatal pathway. The expression of this behavior may involve pallidal GABA. The objective of this series was to test this possibility. Rats were lesioned unilaterally in the medial forebrain bundle with 6-OHDA and also implanted with a cannula guide aimed for the contralateral globus pallidus. One week following surgery experimentation was initiated. Animals were injected with either saline (1 μ l) or muscimol (ng) into the globus pallidus and then with either amphetamine (1 mg/kg) or MK-801 (0.25 mg/kg). Following the systemic injections of drugs, the animals were placed in automated rotation monitors for one hour.

In order to further evaluate the effects of MK-801 on basal ganglia function, rats were prepared with cannulae guides aimed for the striatum. One week following surgery, microdialysis probes were introduced into the striatum through the guides. Following probe insertion the animals were placed in locomotor chambers. Dialysate samples were collected every 20 minutes and assayed for acetylcholine and choline with HPLC procedures. Following baseline stabilization, the animals were injected intraperitoneally with 0.10 or 0.25 mg/kg of MK-801. Locomotor activity counts as well as dialysate samples were collected every 20 min from 2 hours following injection.

C. Major Findings

Amphetamine and MK-801 produced profound increases in rotational behavior ipsilateral to the MFB lesion following saline injections into the globus pallidus contralateral to the lesion. Injections of muscimol (10 ng) into the globus pallidus were effective in preventing, and in some cases reversing, the ipsilateral rotational behavior induced by the systemically administered drugs. These findings suggest that rotational behavior of both MK-801 and amphetamine are mediated by inhibition of GABA transmission in the globus pallidus.

MK-801 administered at 0.25 mg/kg had little effect on locomotor activity during the time interval tested. The animals appeared to be somewhat catatonic. There was also no effect on striatal acetylcholine at this dose. The lower dose (0.10 mg/kg), on the other hand, produced significant increases in locomotor output and concomitant decreases in striatal acetylcholine. This would suggest that locomotor activation through glutamatergic blockade is causal to decreasing acetylcholine function in the striatum or that, under some conditions, acetylcholine interneurons in the striatum are under glutamatergic control.

II. Significance to Biomedical Research and the Program of the Institute

Non-competitive NMDA antagonists produce a constellation of behavioral effects similar to those seen in schizophrenia. Many researchers have considered the drug-induced behavioral alterations in animals by compounds such as phencyclidine to be the best model of schizophrenia. There seems to be controversy, however, regarding the precise mechanisms of action by which these compounds induce their behavioral effects. The purpose of this project is to identify the neurochemical substrate responsible for producing the psychomotor stimulatory actions of noncompetitive NMDA antagonists.

III. Proposed Course of Project

A. The effects of PCP, MK-801 and amphetamine will be evaluated on pallidal GABA with microdialysis procedures to determine if the actions of these three compounds converge on this neurotransmitter system.

B. The mechanisms underlying the ability of MK-801 to alter striatal acetylcholine will be explored.

C. The circuitry underlying PCP- and MK-801-induced locomotor excitation will be explored with lesioning and microinjection procedures.

D. Lesion procedures will be used to ascertain the role of mesolimbic dopamine in mediating the psychomotor stimulatory actions of MK-801 and phencyclidine.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02459-Q4 BP
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mechanism(s) of Action of Carbamazepine in the Central Nervous System of the Rat		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Mike Clark, Ph.D., Staff Fellow, BPB, NIMH Robert M. Post, M.D., Chief, BPB S.R.B. Weiss, Ph.D., Staff Fellow, BPB, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, MD		
TOTAL STAFF YEARS: 1.5	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The phenomenon of <u>contingent tolerance to carbamazepine</u> (CBZ) during amygdala-kindled seizure development was discovered by Dr. Weiss of the Section on Psychobiology. We have begun <u>autoradiographic studies</u> of various receptors to investigate the biochemical mechanism of this contingent tolerance to CBZ. The assays have been performed in a pilot study. The following neurotransmitter receptors were assayed. 1) <u>Glutamate NMDA</u> subtype with [³H]MK-801. 2) <u>GABA_A</u> with [³H]muscimol and [³⁵S]TBPS. 3) <u>Central-type benzodiazepine</u> with [³H]Ro-15-1788. 4) <u>Peripheral-type benzodiazepine</u> with [³H]Ro 5-4864. 5) Adenosine A₁ and A_{2a} subtypes with [³H]cyclohexyladenosine and [³H]CGS-21680, respectively. No changes in striatal adenosine A_{2a} receptor binding were observed. Likewise, no changes in the hippocampus were observed for adenosine A₁ or NMDA receptors. [³H]Ro 5-4864 was not a suitable ligand for autoradiographic analysis of peripheral-type benzodiazepine receptors. However, the data have confirmed that GABA_A receptors and central-type benzodiazepine receptors show increased binding in the dentate gyrus of the hippocampus with electrical kindling of the amygdala. We extend those findings by showing that [³⁵S]TBPS binding is also increased in the dentate gyrus with kindling. Interestingly, in rats made <u>tolerant to CBZ</u> (contingent tolerance), <u>GABA_A receptor binding was decreased</u> toward control levels as compared with non-tolerant kindled rats, while central-type <u>benzodiazepine receptor and TBPS binding did not differ</u> between tolerant and non-tolerant rats. This finding suggests that the mechanism of contingent tolerance to CBZ involves a decrease in GABA_A receptor function without involvement of the benzodiazepine site. This biochemical correlate of GABA_A receptor changes with contingent tolerance to CBZ in the amygdala kindling paradigm is the primary focus of ongoing research. </p>		

I. Project Description

A. Objectives

The Unit on Neurochemistry of the Biological Psychiatry Branch concentrates investigations on the mechanism(s) of action of various anticonvulsants with major emphasis on the tricyclic iminostilbene derivative carbamazepine. The pharmacological actions of carbamazepine in the central nervous system (CNS) are of particular interest due to the numerous therapeutic benefits associated with this drug. Carbamazepine is not only useful as an anticonvulsant, but it also has antimanic and antidepressant properties. By determining the neurochemical systems altered by carbamazepine, insight into the basis of epilepsy and/or affective disorders is anticipated.

Carbamazepine has been shown by several laboratories, including the Unit on Neurochemistry (see Z01 MH 01833-08 BP), to bind to adenosine A_1 receptors in the CNS. Others had reported that carbamazepine bound to both A_1 and A_2 adenosine binding sites. We previously reported that carbamazepine selectively binds to the adenosine A_1 receptor. This was the first major difference discovered for carbamazepine and caffeine interactions with the adenosine receptor system. Until this finding of carbamazepine's selectivity, it seemed paradoxical that both carbamazepine and caffeine could act as adenosine receptor antagonists. Now it appears that the differential pharmacology of carbamazepine (CNS depressant) and caffeine (CNS stimulant) may reside in the selectivity of carbamazepine, but not caffeine, for adenosine A_1 receptors.

The phenomenon of contingent tolerance to the anticonvulsant activity of carbamazepine was discovered (Dr. S.R.B. Weiss, BPB, NIMH). Treating rats with carbamazepine during amygdala-kindled seizure development (when it is ineffective) renders rats unresponsive to the anticonvulsant action on fully developed kindled seizures (when it is effective). Carbamazepine binds to both adenosine A_1 receptors and peripheral-type benzodiazepine receptors (PBR) with similar affinities (within the therapeutic range). Therefore, it was important to test whether carbamazepine altered binding at adenosine A_1 receptors and/or PBR to explain the phenomenon of contingent tolerance to carbamazepine. Adenosine is thought to act as an endogenous anticonvulsant, while PBRs appear to be involved in the kindling process. Since it was reported by others (Shin et al., J. Neurosci. 1985, 5:2696) the GABA_A receptors and central-type benzodiazepine receptors are increased in the dentate gyrus of the hippocampus with kindling, we thought it important to test binding at these two sites in animals made tolerant to the anticonvulsant activity of carbamazepine.

B. Methods Employed

Radioreceptor assays, scintillation counting, receptor autoradiography, quantitative densitometry, spectrophotometry.

C. Findings

Carbamazepine was again shown to be selective for the adenosine A_1 subtype of adenosine binding sites using the highly A_2 selective compound [3H]CGS-21680 while caffeine displaced A_2 binding. These studies were performed in both striatal tissue homogenates (A_2 receptors are limited to the striatum, nucleus accumbens and olfactory tubercle) and by receptor autoradiography. Also, with autoradio-

graphic techniques, carbamazepine was found to be more potent than caffeine in displacing agonist [^3H]CHA and antagonist [^3H]DPCPX from adenosine A_1 binding sites. On the other hand, it is intriguing that carbamazepine was less potent than caffeine at displacing [^3H]NECA binding to adenosine A_1 receptors. These results suggest the possibility for [^3H]NECA labeling a non- A_1 binding site in several brain areas (thalamus, cerebral cortex, hippocampus and cerebellar molecular layer). This non- A_1 or A_{2a} site may be important for the CNS effects of caffeine, but most likely not for carbamazepine.

We have begun a pilot audiographic study of various receptors to investigate the biochemical mechanisms of contingent tolerance to CBZ. The following receptor systems were assayed: 1) Glutamate NMDA subtype with [^3H]MK-801, 2) GABA $_A$ with [^3H]muscimol and [^{35}S]TBPS, 3) Central-type benzodiazepine with [^3H]Ro 15-1788. 4) Peripheral-type benzodiazepine with [^3H]Ro 5-4864. 5) Adenosine A_1 and A_{2a} subtypes with [^3H]cyclohexyladenosine and [^3H]CGS-21680.

No changes in striatal adenosine A_{2a} receptor binding were observed. Likewise, no changes in the hippocampus were observed for adenosine A_1 or NMDA receptors. [^3H]Ro 5-4864 was not a suitable ligand for audiographic analysis of peripheral-type benzodiazepine receptors. However, the data have confirmed that GABA $_A$ receptors and central-type benzodiazepine receptors show increased binding in the dentate gyrus of the hippocampus with electrical kindling of the amygdala. We extend those findings by showing that [^{35}S]TBPS binding is also increased in the dentate gyrus with kindling. Interestingly, in rats made tolerant to CBZ (contingent tolerance), GABA $_A$ receptor binding ([^3H]muscimol) was decreased toward control levels as compared to non-tolerant kindled rats, while neither central-type benzodiazepine receptor nor [^{35}S]TBPS binding differed between tolerant and non-tolerant rats. This finding suggests that the mechanism of contingent tolerance to CBZ involves a decrease in GABA $_A$ receptor function without involvement of the benzodiazepine or TBPS sites. This biochemical correlate of GABA $_A$ receptor changes with contingent tolerance to CBZ in the amygdala kindling paradigm is the primary focus of ongoing research.

II. Significance to Biomedical Research and the Program of the Institute

The significance of the overall project lies in the potential for discovering differential effects of carbamazepine and caffeine on the central adenosine system. These differences will help explain the mechanism(s) of anticonvulsant activity of carbamazepine in the brain and further characterize the possible role of brain adenosine as an endogenous anticonvulsant. Discovering the mechanism of contingent tolerance to carbamazepine could lead to a better understanding of the underlying mechanisms of kindling and to better methods of epilepsy treatment. It appears that carbamazepine's anticonvulsant properties may involve the GABA $_A$ receptor system.

III. Proposed Course of the Project

These studies should continue for at least three years.

PUBLICATIONS

Clark, M., Post, R.M. Carbamazepine, but not caffeine, is highly selective for adenosine A1 binding sites. Eur J Pharmacol, 1989, 164:399-401.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02460-04 BP
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Animal Models of Epilepsy: Molecular Substrates		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Mike Clark, Ph.D., Staff Fellow, BPB, NIMH <div style="display: flex; justify-content: space-between;"> <div> Robert M. Post, M.D., S.R.B. Weiss, Ph.D., </div> <div> Chief, Staff Fellow, </div> <div> BPB, NIMH BPB, NIMH </div> </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, MD		
TOTAL STAFF YEARS: 2.8	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Regional expression of the <u>proto-oncogene c-fos</u> was mapped by <u>in situ</u> hybridization in rat brain sections from animals with <u>CRH-induced</u>, <u>cocaine-induced</u>, and <u>amygdala-kindled seizures</u>. Studies on the interactions of acute <u>benzodiazepine</u> and <u>chronic caffeine</u> (CAF) treatment on the induction of <u>c-fos mRNA</u> by a CAF challenge was investigated in rats. These findings were reported in previous annual reports on this research project and are published or submitted for publication.</p> <p>Studies on the regional expression of peripheral-type benzodiazepine receptor (PBR) <u>mRNA</u> in rat brain were continued. Although these studies are only in the preliminary stages, it appears that <u>cocaine</u> treatment may <u>decrease</u> PBR <u>antisense</u> mRNA without affecting the sense strand. The effects of lidocaine and other drugs altering CNS excitability will be investigated as well. However, difficulties were encountered in the methodology of <u>in situ</u> hybridization for the PBR mRNA. These technical problems are almost resolved to allow this study to continue.</p> <p>Studies on the expression of mRNAs and proteins for <u>glucocorticoid</u> and <u>mineralocorticoid</u> receptors with electrical kindling of the amygdala are to be started.</p>		

I. Project Description

A. Objectives

The proto-oncogene *c-fos* is rapidly (within 15 minutes) and transiently expressed in many cell types following various stimuli and belongs to a class of genes called "immediate-early genes". The use of *in situ* hybridization for *c-fos* mRNA and immunohistochemistry for Fos protein are techniques recently applied to map potential brain structures activated during various means of CNS stimulation. The phenomenon of kindling is a permanent process in which daily electrical stimulation of the amygdala (or other limbic system structures) with an initially subconvulsant stimulus gradually leads to the production of major motor seizures. The progression of the kindling process is manifested in several distinct stages beginning with brief focal afterdischarges and culminating in full-blown motor convulsions. The progressive nature of the kindling process involves propagation of the evoked response from the primary site to secondary sites.

The biochemical alterations eliciting the growth and spread of afterdischarges and accompanying the behavioral seizure stages of kindling are not known. A plethora of potential protein changes can be envisioned to occur during this development of altered responsiveness. However, the mechanism by which protein changes would be evoked is not well understood. A possible explanation is the regulation of the genome, and subsequently, protein synthesis, by extracellular stimuli associated with neuronal activity during kindling evolution.

Recently, a schema for rapid regulation of gene expression was proposed by Morgan and Curran (*Trends Neurosci* 12). They suggested a stimulus-transcription coupling cascade in which the protein products of the immediate-early genes, *c-fos* and *c-jun*, act as "third messenger molecules" to couple extracellular stimuli to target genes in the nucleus. Several investigators established that *c-fos* mRNA and its protein product Fos are dramatically increased in rat brain following long-term potentiation, afterdischarges, kindling, seizures and various other means of neuronal excitation (see Z01 MH 01833-08 BPB). It is possible that expression of the *c-fos* gene is involved in a cascade of events necessary for the progression to kindled seizures. In the present investigation, we used *in situ* hybridization for *c-fos* mRNA to map potential neuronal structures activated during the various stages of amygdala kindling. This study was an attempt to determine the brain regions recruited in the evolution of general convulsions using electrical and pharmacological kindling paradigms.

In humans, serum cortisol levels are elevated after seizures, and corticosteroid drugs have found to be useful in the treatment of some seizures (Pritchards, *Epilepsia* 32:S46, 1991; Holmes, *Epilepsia* 32:S11, 1991). Therefore, the possibility exists for involvement of steroid receptors in seizure activity. We plan to examine whether electrical kindling of the amygdala alters glucocorticoid or mineralocorticoid receptor binding or mRNA levels (no data generated yet).

B. Methods Employed

In situ and Northern hybridizations, receptor autoradiography, animal seizure paradigms (cocaine kindling and electrical kindling of the amygdala), brain tissue sectioning with cryostat.

C. Major Findings

1. Cocaine-Kindled Seizures

All rats receiving cocaine showed stereotypic movements characteristic of cocaine-induced motor stimulation. However, only about one-half of the rats had seizures. No behavioral changes were apparent in saline-treated control animals.

Visualization of c-fos mRNA expression due to cocaine-induced seizures was carried out by *in situ* hybridization. The pattern of c-fos induction in acute and kindled cocaine seizures was identical. In saline injected control rats, the expression of c-fos mRNA was very low in all brain areas. The expression of c-fos in animals that received cocaine but did not have a seizure (i.e., only stereotypy) was no different from saline controls. However, the rats that did convulse to cocaine showed a large increase of c-fos mRNA content in several brain regions. The granular layers of both the dentate gyrus and olfactory bulb showed the largest cocaine-induced c-fos expression. This was followed by moderate increases in the piriform cortex, entorhinal cortex, olfactory tubercle and anterolateral amygdalohippocampal area. c-Fos mRNA was also elevated to a lesser degree in the lateral septal nuclei, accumbens nucleus, caudate-putamen as well as outer and inner layers throughout the cerebral cortex. No changes were observed in the cerebellum.

2. Caffeine-Induced Seizures

Studies on the interaction of acute benzodiazepine (BZ) and chronic caffeine (CAF) treatment on the induction of c-fos mRNA by a CAF challenge were conducted in rats. Acute CAF-induced c-fos was limited to the striatum and olfactory tubercle in the absence of seizures. However, with a CAF-induced seizure, c-fos was also elevated in the hippocampus and olfactory bulb. Acute pretreatment with Ro 5-4864 (an agonist selective for the peripheral-type benzodiazepine receptor) or Ro 15-1788 (an antagonist selective for central-type benzodiazepine receptors) had no effect on CAF-induced c-fos in the absence of seizure. However, with CAF-induced seizure, Ro 5-4864 (but not Ro 15-1788) potentiated CAF-induced c-fos in striatum, hippocampus and olfactory bulb and also significantly increased c-fos in cortical regions. Chronic CAF treatment reduced basal levels of c-fos with no effect on the amount of striatal c-fos induced by a CAF challenge. Withdrawal from CAF restored striatal c-fos levels to control levels. These findings suggest that CAF-induced c-fos and CAF-induced seizures may involve an interaction with the peripheral-type benzodiazepine receptor system.

3. Electrical Kindling of the Left Amygdala

a. Sham Controls and Rats with no Afterdischarge:

Basal expression of c-fos mRNA, as determined from sham control rats (n=14), was low and limited to the piriform cortex, pyramidal cell layer of the hippocampus and granule cells of the dentate gyrus. In six of seven rats that were stimulated from 1 to 28 days but showed no afterdischarges (AD), there was no increase in c-fos mRNA as compared with sham controls. In one rat stimulated one

day but showing no AD, there was increased c-fos expression unilaterally, ipsilateral to the stimulated side, in the piriform, occipital and entorhinal cortices.

b. Stages 1 and 2

In rats that were sacrificed 15 minutes after scoring stage 1 or 2 according to the scale of Racine, there were two basic patterns of c-fos induction. One pattern involved unilateral increases of c-fos in cerebral cortical areas (piriform, insular, perirhinal, entorhinal, occipital, and parietal) ipsilateral to the stimulated side. The areas showing enhanced c-fos mRNA expression were most marked in the piriform, occipital, and entorhinal cortices. The rats with this first distribution pattern had AD durations averaging 29 ± 10 sec. (mean \pm SEM, $n=7$). The second pattern of c-fos expression in rats at stage 1 or 2 exclusively involved the granule cell layer of the dentate gyrus and the pyramidal cell layer of the hippocampus. The c-fos increases in these animals were either unilateral (ipsilateral to stimulated side) or bilateral. The AD duration for this second group averaged 61 ± 5 sec (mean \pm SEM, $n=12$) and was statistically longer than that for the first group ($p < 0.05$, t-test). There was no difference in the AD duration for a unilateral or bilateral effect in the "hippocampal" group. Three other rats that were having afterdischarges (i.e., stage 1 with 1, 4, or 21 days of stimulation) had no increased c-fos expression in any brain region. Two rats that had AD durations of about 50 sec for 11 days showed bilateral hippocampal (including dentate gyrus) and entorhinal cortical increases of c-fos mRNA expression.

c. Stage 3

The c-fos expression in animals at stage 3 involved both the cortical regions and the hippocampus (including the dentate gyrus). However, the cortical increases were not consistently unilateral (often bilateral) while the dentate gyrus always showed bilateral expression of c-fos mRNA.

d. Stages 4 and 5

At stages 4 and 5, distribution of c-fos mRNA appeared to be a combination of the two patterns described for rats at stages 1 and 2 except that the expression was nearly always bilateral in all brain regions ($n=16$). However, one rat at stage 4 and two rats at stage 5 had no expression of c-fos mRNA in the hippocampus while showing bilateral cortical expression with the exception of contralateral induction in the piriform cortex.

e. Running Fits

Rats exhibiting running fits associated with their stage 5 seizures had c-fos increases in the dorsal aspect of the inferior colliculus (bilaterally) in addition to the other brain regions described above for stages 4 and 5. The increase in the inferior colliculus was limited to animals with running fits. It is thought that the inferior colliculus is critical for running fits. Therefore, *in situ* hybridization of c-fos mRNA can provide a map of neuronal structures important for various types of seizures.

f. Correlation of Hippocampal c-fos with Afterdischarge Duration

A direct correlation of c-fos induction in the hippocampus with afterdischarge duration was observed. Longer afterdischarge durations were required for hippocampal c-fos induction at all seizure stages. The afterdischarge duration was from 1.6 to 4.5 times longer in animals exhibiting hippocampal c-fos induction. This difference was highly significant ($p < 0.0001$).

4. Peripheral-type Benzodiazepine Receptor mRNA Studies

A probe of the peripheral-type benzodiazepine receptor (PBR) mRNA was recently developed. Studies on the regional expression of PBR mRNA in rat brain were started. These studies are only in the preliminary stages and indicate that the endogenous antisense strand may regulate the expression of PBR protein. Studies designed to investigate this potential unusual regulatory mechanism are based on preliminary results suggesting that acute cocaine treatment of rats decreases the antisense strand, whereas chronic cocaine use (humans) was reported (J Clin Psychiatry 51:10, 1990) by others to increase the density of PBR protein on platelets. We have encountered methodological difficulties using the probe for PBR mRNA. However, these problems are nearly remedied to allow this study to continue.

II. Significance to Biomedical Research and the Program of the Institute

The results from these studies suggest that kindling may involve a permanent regulation of the genome by external stimuli. These studies prompt the investigation of other genes for altered expression during the kindling process. The enkephalin and dynorphin genes are of specific interest since c-fos has been linked to the subsequent expression of these genes. Also, studies by the Section on Psychobiology suggest that the peripheral-type benzodiazepine receptor (PBR) might be involved in the phenomenon of amygdala kindling. We plan to test whether expression of PBR mRNA may be altered with kindling. Discovering molecular and biochemical changes associated with kindling may give insight into the phenomena of learning and memory and neuronal plasticity. These investigations may prove important in understanding the substrates of seizures and affective disorders.

III. Proposed Course of the Project

These studies should continue for at least three years.

Publications

Clark M, Post RM, Weiss SRB, Cain CJ, Nakajima T. Regional expression of c-fos mRNA in rat brain during the evolution of amygdala-kindled seizures, Mol Brain Res 1991; 11:55-64.

Clark M, Post RM, Weiss SRB, Nakajima T. Expression of c-fos mRNA in acute and kindled cocaine seizures in rats, Brain Res 1992; 582:101-106.

Clark M, Weiss SRB, Post RM. Expression of c-fos mRNA in rat brain after intracerebroventricular administration of corticotropin-releasing hormone, Neurosci Lett 1991; 132:235-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02461-04 BP
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacology of Adenosine and Peripheral-type Benzodiazepine Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Mike Clark, Ph.D., Pharmacology Research Associate BPB, NIMH Robert M. Post, M.D. Chief BPB, NIMH S.R.B. Weiss, Ph.D. Staff Fellow BPB, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Neurochemistry		
INSTITUTE AND LOCATION NIMH, Bethesda, MD		
TOTAL STAFF YEARS: 2.5	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Lidocaine, carbamazepine, and valproic acid were found to have greater potency for displacing [³ H]Ro5-4864 than [³ H]PK-11195 at the PBR in olfactory bulb. These results suggest that the anticonvulsant or convulsant effects of these drugs may involve a PBR component. [³ H]PK-11195 binding in the olfactory bulb revealed <u>increased density</u> and decreased affinity of <u>PBR</u> in amygdala-kindled rats receiving multiple stage 5 seizures compared with sham control rats 24 hr after the last seizure. We plan to test PBR binding in other brain regions (e.g., hippocampus, cerebral cortex, striatum, cerebellum) to determine whether kindling alters this system in selective brain regions. It was recently reported by others that the PBR are involved in stereogenesis by increasing cholesterol transport into mitochondria. Preliminary data in our laboratory suggest that <u>carbamazepine</u> also <u>increases cholesterol uptake</u> by rat brain mitochondria with resultant increased cholesterol metabolism (presumably steroid production). Since carbamazepine binds with PBR, we hypothesize that (and plan to determine whether) carbamazepine does increase steroid synthesis in rat brain. This may be an important effect of the CNS effects of carbamazepine. It was found that <u>sulfonated dyes</u> , Fast Green FCF and Brilliant Blue FCF, <u>stimulated poly (ADP-ribosylation)</u> in rat brain <u>nuclei</u> by a mechanism that appears to be devoid of DNA damage. The structurally related dye Light Green SF Yellowish was inhibitory by itself and dose-dependently inhibited the stimulatory effects of Fast Green FCF. The sulfonated dyes may become important tools for elucidating the mechanism of action of the chromatin bound enzyme poly(ADP-ribose) polymerase.		

I. Project Description

A. Objectives

Drs. Weiss and Post have reported differential effectiveness of carbamazepine in the developmental and completed stages of amygdala-kindled and local anesthetic-kindled seizures in rats. Since carbamazepine has been shown to bind at clinically relevant concentrations to brain adenosine and peripheral-type benzodiazepine (PBR) receptors (see Z01 MH 01833-04 BP), the binding of local anesthetics cocaine and lidocaine to PBR was tested. These studies have been extended by starting experiments to investigate whether cocaine or lidocaine affect expression of the mRNA for the PBR (see Z01 MH 02460-04 BP).

Altered thyroid status regulates adenosine receptors and catalytic activity of adenylate cyclase (AC) in adipocytes. Adenosine has been shown to modulate the release of adrenocorticotrophic hormone from cultured anterior pituitary cells. Therefore, we postulated that central adenosine receptors may be altered during altered thyroid activity in the rat. This hypothesis is currently being investigated. However, Dr. Stein (the major contributor to that work) has moved to the University of Manitoba where he is continuing this project in collaboration with us.

Adenosine receptors are classified as A_1 (inhibits AC activity) and A_{2b} (stimulates AC activity) and A_{2a} (function unknown). A third subtype of adenosine receptor has been suggested to involve calcium fluxes and has been tentatively termed the A_3 receptor. We have found that adenosine A_1 binding is inhibited by NAD under certain conditions and feel that this may represent a new class of adenosine binding sites, possibly the A_3 receptor.

Specific enzymes in the brain can stimulate the transfer of ADP-ribose from NAD to proteins. The physiological significance of this process, referred to as ADP-ribosylation, is not well understood. During the past year Dr. Clark discovered a compound (Fast Green FCF) that differentially modulates agonist binding to adenosine A_1 and A_2 receptors and that inhibits binding to PBR. During the course of that work, it was found that stereospecific [3H]NAD binding was markedly potentiated by the dye. This labeling of brain tissue with [3H]NAD was subsequently found to represent ADP-ribosylation. Since the ADP-ribosylation is known to alter several normal processes in brain function (such as Ca^{2+} homeostasis, DNA repair, transcription, signal transduction via GTP-binding protein), characterization of the Fast Green FCF-induced ADP-ribosylation and determination of its physiological consequences were investigated.

It was recently reported by others that a function of the mitochondrial PBR may be to increase cholesterol uptake by mitochondria. Subsequently, cholesterol is converted to steroid precursors, pregnenolone and progesterone. Since carbamazepine is known to bind to the PBR at therapeutically relevant concentrations, we have started to investigate whether carbamazepine alters steroidogenesis in rat brain mitochondria.

B. Methods Employed

Radioreceptor assays, scintillation counting, receptor autoradiography, quantitative densitometry, spectrophotometry, rat models of hypo- and hyper-thyroidism

(diet), gel electrophoresis of proteins, ADP-ribosylation, HPLC, fraction collection, differential centrifugation to isolate subcellular fractions.

C. Major Findings

Both lidocaine and tetracaine dose-dependently inhibited binding at the PBR in rat olfactory bulb. However, lidocaine was substantially more potent in this effect. The IC_{50} values for lidocaine and tetracaine were 355 nM and 302 μ M, respectively. Therefore, lidocaine was approximately 1000-fold more potent than tetracaine. Procaine did not inhibit binding until a concentration of 1 mM was used. This high concentration of procaine caused only a 31% inhibition. Cocaine inhibited binding by only 13%, which required 1 mM cocaine. Lidocaine effectively blocked all PBR binding at 10 μ M concentration. The anticonvulsant drug sodium valproate totally lacked any inhibitory actions at concentrations up to 1 mM. However, the anticonvulsant drug carbamazepine also dose-dependently inhibited PBR binding with an IC_{50} of 51 μ M and complete displacement at 560 μ M.

The data clearly show that lidocaine potently inhibits agonist [3 H]Ro 5-4864 binding at the PBR. The effect is not characteristic of all local anesthetics since cocaine and procaine were virtually without effect. In one experiment, lidocaine and cocaine were tested against PBR antagonist [3 H]PK-11195 binding and the results were virtually identical to that for agonist [3 H]Ro 5-4864 binding. In autoradiographic analysis of [3 H]Ro 5-4864 and [3 H]Ro 15-1788 binding in rat brain, lidocaine specifically blocked binding at the PBR and had no effect on the binding of [3 H]Ro 15-1788 to central-type benzodiazepine receptors. Cocaine had no effect on the binding of either ligand.

Lidocaine, carbamazepine and valproic acid were tested as inhibitors (dose-responses) of agonist [3 H]Ro 5-4864 and antagonist [3 H]PK 11195 binding in olfactory bulb homogenates. It was found that each of these compounds had greater affinity for the agonist subsite (Ro 5-4864) of the PBR.

[3 H]PK 11195 binding in the olfactory bulb revealed increased density and decreased affinity of PBR in amygdala kindled rats receiving multiple stage 5 seizures compared to sham control rats 24 hr after the last seizure. We plan to test PBR binding in other brain regions (e.g., hippocampus, cerebral cortex, striatum, cerebellum) to determine whether kindling alters this system in selective brain regions.

It was recently reported by others that the PBR are involved in steriogenesis by increasing cholesterol transport into mitochondria. Preliminary data in our laboratory suggest that carbamazepine also increases cholesterol uptake by rat brain mitochondria with resultant increased cholesterol metabolism (presumably steroid production products have not yet been identified). Since carbamazepine binds to PBR, we hypothesize that (and plan to determine whether) carbamazepine does increase steroid synthesis in rat brain. This may be an important part of the CNS effects of carbamazepine.

The regulation of central adenosine and β -adrenergic receptors by alterations in thyroid function in rats is also being investigated. Preliminary results indicate that hyperthyroidism may down-regulate the number of striatal adenosine A_2 binding sites with no alteration of β -adrenergic receptors. Preliminary results

also indicate that hypothyroidism may decrease cerebral cortical β -adrenergic receptors. These studies await further analysis due to the relocation of Dr. Stein (the major contributor to that work) has moved to the University of Manitoba where he is continuing this project in collaboration with us.

Studies on identifying the hypothesized central adenosine A_2 receptor were started. A small component of apparently A_1 binding is displaced by nicotinamide adenine dinucleotide (NAD) which was previously shown by others not to bind at adenosine receptors. [3 H]NAD binding was shown to be stereospecific by autoradiographic analysis. [3 H]NAD binding is partially inhibited by adenosine agonists and by carbamazepine. These preliminary results hint at the possibility of an unidentified adenosine receptor which also recognizes NAD. These studies have led to the discovery of an endogenous ADP-ribosylation caused by a sulfonated dye (Fast Green FCF) that was found to differentially alter agonist binding to adenosine A_1 and A_2 receptors. Fast Green FCF stimulated adenosine A_1 binding modestly (up to 20% at 1mM), while it dose-dependently inhibited adenosine A_2 binding (up to 42% at 1mM). Moreover, Fast Green FCF dose-dependently inhibited PBR binding almost completely but had no effect on binding at the central-type benzodiazepine receptor. The effect of Fast Green FCF on PBR binding was potentiated by $MgCl_2$. It was found that Fast Green FCF dose-dependently stimulated the transfer of ADP-ribose from NAD to brain proteins (ADP-ribosylation). This effect of the dye is markedly potentiated by $MgCl_2$. Protein gel electrophoresis of ADP-ribosylated proteins revealed two major bands with molecular weights of about 117 kDa and 10 kDa. The stimulatory effects of Fast Green FCF on ADP-ribosylation was highly localized to the purified nuclear fraction. Enzymatic digestion of the product coupled with HPLC analysis conclusively showed that Fast Green FCF stimulated the nuclear enzyme poly(ADP-ribose) polymerase to produce polymers of poly(ADP-ribose) from NAD. Since this enzyme is activated by drugs and procedures (e.g., radiation) that cause DNA damage, it was tested whether Fast Green FCF caused DNA damage as a potential mechanism for its stimulation of poly(ADP-ribosylation). However, the dye did not appear to elicit DNA damage, and its mechanism of stimulation may prove important to the understanding of poly(ADP-ribose) polymerase activation. The mechanism of dye-induced poly(ADP-ribosylation) requires further investigation.

II. Significance to Biomedical Research and to the Program of the Institute

The finding that lidocaine binds with high affinity to PBR suggests that its anticonvulsant and antiarrhythmic activity may involve the PBR. These possibilities are being investigated. The role of the central adenosine receptor system in altered thyroid status may help the understanding of the basis for hypo- and hyper-thyroid states in humans, as well as assist the overall knowledge of adenosine receptor functions. The continued study of different types of adenosine receptors will further the understanding of the role of this nucleotide as a neuromodulator and has implications in many CNS disorders such as the epilepsies, and in the ischemic insults to the brain associated with stroke. Studies on the Fast Green FCF-stimulated poly(ADP-ribosylation) could lead to a better understanding of the physiological significance of poly(ADP-ribosylation) reactions in the brain, such as potential involvement in transcription.

III. Proposed Course of the Project

These studies should continue for at least three years.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02523-03 BP
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Second Messenger Systems in Behavioral Sensitization and Kindling		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Jeffrey Rosen, Ph.D.		
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Neurochemistry		
INSTITUTE AND LOCATION NIH, Bethesda, MD		
TOTAL STAFF YEARS: .5	PROFESSIONAL: .5	OTHER: .0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The intention of this project is to examine post-synaptic changes in <u>second and third messenger systems</u> in brain areas involved in behavioral sensitization to cocaine or kindling. The role of dopamine-regulated phosphoproteins (i.e., DARPP-32) in the striatum during behavioral sensitization is being studied. Preliminary results suggest that the increases in the phosphorylation of DARPP-32 seen with acute cocaine administration are attenuated following repeated administration of cocaine. FOS, a transcription factor that acts as a third messenger, also behaves in a similar fashion. In the striatum, the increases in FOS seen after an acute cocaine injection are diminished following repeated administration of cocaine. Because both DARPP-32 and FOS are regulated by the dopamine D1 receptor in the striatum, a common, but as yet unknown, mechanism may be responsible for the similar changes seen in both systems.</p> <p>With cocaine seizures, FOS is also increased in the hippocampus. This does not seem to be related to whether the cocaine was given acutely or repeatedly, but only to the incidence of the seizure. Thus, different brain regions seem to participate in cocaine sensitization and seizures.</p> <p>In other experiments related to fear conditioning and behavioral sensitization, rats that are given foot shocks or are reintroduced to an environment in which they have previously been given footshocks, show increased startle response. In these situations, c-fos mRNA is also dramatically increased in the amygdala. This is the first study to show that following unconditioned and conditioned fear, c-fos is increased in the amygdala, a nucleus known to be crucial for fear conditioning and sensitization.</p>		

I. Project Description

A. Objectives

The goal of the project is to elucidate second and third messenger system alterations that occur during behavioral sensitization to cocaine and kindling.

B. Methods Employed

1. Subjects

Sprague-Dawley rats weighing 200-350g housed in group cages.

2. Procedure

a. Behavioral sensitization - rats are given either cocaine or saline for 1 to 4 days while their locomotor activity is monitored. One, three or seven days later all rats are tested for locomotor activity with cocaine or saline. Following the test, the rats are sacrificed and the brains removed. Various biochemical, immunohistochemical and molecular biological assays for phosphoproteins, kinases or mRNAs or proteins are performed.

b. Kindling - Rats are either electrically or chemically kindled. Following seizures, the same assays mentioned above are performed.

C. Major Findings

Behavioral sensitization: Preliminary results suggest that the phosphorylation of the dopamine-related phosphoprotein DARPP-32 in the striatum is altered with cocaine. Acute cocaine injections increase the amount of phosphorylated DARPP-32. Following repeated injections of cocaine, the levels of phosphorylated DARPP-32 appear to return to near baseline levels. Although the results are preliminary and somewhat variable, they suggest that adaptation occurs in a dopamine-related phosphoprotein. More studies with different tissue preparation procedures are needed to draw firm conclusions.

Levels of c-fos mRNA and FOS protein also seem to be increased in the striatum following repeated cocaine administration and decreased with repeated administration. Thus, similar changes appear to be induced in both second and third messenger systems concomitant with behavioral sensitization. What is particularly interesting is the possibility that with repeated administration compensatory biochemical mechanisms become activated to reduce the initial increases seen following acute administration, while at the same time behavioral sensitization develops.

Kindling: Cocaine kindling has been shown by Clark et al. to increase the expression of c-fos mRNA in the hippocampus. Our results show that with acute and repeated cocaine administration which induce behavioral sensitization but not seizures, FOS is not increased in the hippocampus. Acute cocaine-induced seizures also increase FOS levels in the hippocampus. Thus, increased FOS expression following cocaine can occur in two distinct anatomical systems which correlates with the behavioral outcome. Increases in the hippocampus are associated with seizures, while increases in the striatum are associated with behavioral sensitization.

With another form of sensitization, contextual fear conditioning, c-fos has been shown to dramatically increase in the amygdala. Because the amygdala is known to be crucial for fear conditioning and possibly sensitization, this finding that c-fos is expressed under these conditions may be important for understanding the neuroanatomical and molecular substrates of sensitization.

II. Significance to Mental Health Research and Program of the Institute

The mechanisms of sensitization to cocaine or kindling are not well understood. However, second and third messenger systems in distinct anatomical loci probably play a significant role in these processes. Distinction between anatomical systems involved behavioral sensitization and seizures may be important for elucidation of the progression from a behavioral sensitized state to a seizure state. Knowledge about biochemical differences between acute and repeated cocaine administration is fundamental for our understanding of biochemical and molecular mechanisms underlying sensitization processes.

III. Proposed Course of Project

This project should take two to three years to complete.

Publications

Campeau S, Hayward MD, Hope BT, Rosen JB, Nestler, EJ, Davis M. Induction of the c-fos protooncogene in rat amygdala during unconditioned and conditioned fear, Brain Research 1991; 565:349-52.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02524-03 BP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) c-Fos and Peptide mRNA Expression in Kindling		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Jeffrey Rosen, Ph.D.	BPB, NIMH	
Mike Clark, Ph.D.	BPB, NIMH	
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Neurochemistry		
INSTITUTE AND LOCATION NIH, Bethesda MD		
TOTAL STAFF YEARS: .75	PROFESSIONAL: .25	OTHER: .50
CHECK APPROPRIATE BOXES)		
<input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)		
<p>Previous work in this Branch has elucidated the neuroanatomical pattern of c-fos mRNA expression with the development of amygdala kindled seizures. This project extends that line of investigation to the expression of mRNAs of peptides during kindling and their relationship to c-fos induction. mRNA levels of enkephalin, dynorphin, and thyrotropin releasing hormone (TRH) were measured by <u>in situ</u> hybridization at different stages of kindling. Enkephalin and TRH mRNAs increase, but dynorphin mRNA decreases with kindling. Each has a different pattern of activation or inhibition at the various stages of kindling. For instance, enkephalin mRNA increases in the entorhinal cortex after a stage 1 seizure and in the pyriform and entorhinal cortices after a stage 5 seizure, while TRH increases in both the pyriform and entorhinal cortices following stage 1 and 5 seizures. TRH also increases in the dentate gyrus. Dynorphin decreases in the dentate gyrus after stage 5. Changes in TRH and dynorphin are transient, and only enkephalin mRNA in the pyriform cortex remains elevated for at least two weeks. Interestingly, the pattern of TRH mRNA expression most closely resembles that of s-fos. This suggests that c-fos and TRH expression in kindling may be related and co-localized in certain populations of cells. Indeed, in situ double labeling of TRH mRNA with an oligonucleotide probe, and FOS protein and FOS-related antigens with an antibody demonstrates that the vast majority of FOS labelled cells are labelled with TRH mRNA following a kindled seizure. This suggests that FOS may act as a transcription factor for TRH during kindling.</p>		

I. Project Description

A. Objectives

The goal of this project is to study the neuroanatomical patterns of mRNA induction of several peptides in various stages of amygdala kindled seizures and their relationship to c-fos induction in kindling. Expression of c-fos and peptides in the same cells following kindling may indicate that their expression is temporally and functionally linked.

B. Methods Employed

1. Subjects

Sprague-Dawley rats weighing 250-350g housed in group cages.

2. Procedure

Electrodes are implanted in the amygdala. Following recovery, rats are stimulated once daily to induce kindled seizures. At various stages of seizure development they are sacrificed and the brains sectioned. Oligonucleotide probes of mRNA of the various peptides in question are hybridized in situ and the pattern and extent of mRNA expression are analyzed. Double labeling with probes for mRNAs and antibodies for the FOS protein are also performed and analyzed.

C. Major Findings

The findings thus far have indicated that the expression of peptide mRNA is both spatially and temporally varied. TRH mRNA is increased in the pyriform and entorhinal cortices 24 hours after stage 1 and 5 seizures, while enkephalin mRNA is elevated in the entorhinal cortex after stage 1 seizures and in the pyriform and entorhinal cortices after a stage 5 seizure. In the pyriform cortex, enkephalin mRNA remained elevated for at least two weeks. TRH mRNA is also increased ipsilaterally to stimulation in the pyriform, entorhinal and perirhinal cortices or bilaterally in the dentate gyrus after stage 1 and bilaterally in all of these areas after stage 5. Dynorphin mRNA is decreased in the dentate gyrus 24 hours after a stage 5 seizure. Thus, the patterns of peptide mRNA expression with kindling are complex. However, it suggests that numerous peptides may contribute to seizures in different brain regions and at different stages in the process of kindling. In relation to c-fos, TRH mRNA is most promising to study because its spatiotemporal distribution in kindling is similar to that of c-fos. Indeed, TRH mRNA and FOS-like immunoreactivity are co-localized in the dentate gyrus and pyriform, entorhinal and perirhinal cortices following kindled seizures, suggesting that FOS may act as a transcription factor for TRH.

II. Significance to Mental Health Research and Program of the Institute

A long-term interest of the branch has been the use of the kindling model of epilepsy to investigate the process of sensitization in the nervous system. The analysis of mRNA induction allows one to examine both spatial and temporal molecular changes that occur during the kindling process. The results of co-localization of TRH mRNA and FOS-like immunoreactivity following kindled seizures in limbic structures known to be important for kindling, may indicate an early sequence

of molecular events that may be important for the long-lasting behavioral changes associated with kindling.

III. Proposed Course of Project

The next phase of the project will be continuation of the investigation of the association between early and late molecular changes that may be important for kindling. The project should take about one to two more years to complete.

Publications

Rosen JB, Cain CJ, Weiss SRB, Post RM. Alterations in mRNA of enkephalin, dynorphin, and thyrotropin releasing hormone during amygdala kindling: an in situ hybridization study, Molecular Brain Research (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02525-03 BP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) C-Fos Activation by Panicogenic Drugs		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Jeffrey Rosen, Ph.D., BPB, NIMH David Fontana, Ph.D., BPB, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Neurochemistry		
INSTITUTE AND LOCATION NIH, Bethesda MD		
TOTAL STAFF YEARS: .75	PROFESSIONAL: .25	OTHER: .50
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01532-14 BP
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Catecholamine Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Christopher Hough, Ph.D., Chemist, BPB, NIMH De-Maw Chuang, Ph.D., Unit Chief, BPB, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, Bethesda, MD		
TOTAL STAFF YEARS: 1.2	PROFESSIONAL: 1.2	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The objective of this project is to gain insight into the mechanisms underlying the changes in expression of the <u>β-adrenergic receptors</u> induced by various treatments. We have continued to probe the mechanisms of <u>agonist</u> and <u>antagonist</u> induced changes in β-adrenergic receptor (β-AR) mRNA levels in C₆ glioma cells. In this, we have found that pretreatment with a microtubules disrupter can block agonist-induced down-regulation of β_1-AR mRNA while enhancing the down-regulation of β_2-AR mRNA. The implied differences in the way the two β-AR subtypes are regulated in C₆ glioma cells were supported by experiments using protein synthesis inhibitor, cycloheximide. Thus, microtubule disrupting agents can be used as a tool for clarifying aspects of the changes induced by agonists and antagonists. We have extended our investigation of the interaction between <u>serotonergic</u> and β-adrenergic receptor systems at the level of mRNA expression and continue to find promising evidence that there is cross-regulation between serotonin receptors and β-AR mRNA levels. We have also initiated a study of possible involvement on the <u>peripheral benzodiazepine receptor</u> in the action of carbamazepine to increase the expression of β_2-AR mRNA and protein. The preliminary findings indicate that this induction might be mediated by mitochondrial neurosteroid biosynthesis. </p> <p> In view of the actions of a number of drugs used in the treatment of depression on the serotonergic and β-adrenergic receptor systems, we hope that a better understanding of the mechanisms regulating the expression of the β-AR will offer insight into the nature and effective treatment of this mental disorder. </p>		

I. Project Description

A. Objectives

Our objective has been to gain a better understanding of the mechanisms underlying the changes in expression of the β -adrenergic receptor induced in C_6 glioma cells by various treatments. These treatments include the action of β -adrenergic agonists, antagonists, and some antidepressants.

B. Major Findings

In an extension of our study of the role of microtubules in the regulation of β -adrenergic receptor (β -AR) mRNA, we observed that pretreatment with colchicine, a disrupter of microtubule structure, can dramatically alter the changes in β -AR mRNA levels induced by isoproterenol stimulation. β_2 -AR mRNA down-regulation induced by isoproterenol was enhanced while β_1 -AR mRNA down-regulation was completely blocked causing β_1 -AR mRNA to increase at 1 hour and remain high for up to 4 hours. This was not a general effect since neither isoproterenol nor colchicine affected β -actin mRNA or total cellular RNA levels. Thus, the normal down-regulation of β_1 -AR mRNA induced by isoproterenol requires the presence of microtubules. The up-regulation of β_1 -AR mRNA can be shown to be mediated by the cAMP response to element present in the promoter region of the β_1 -AR gene. This result suggested to us that β_2 -AR mRNA down-regulation induced by isoproterenol is transcriptionally mediated via a repression factor induced by agonist stimulation. Data obtained from C_6 glioma cells receiving a protein synthesis inhibitor, cycloheximide, in the presence or absence of isoproterenol support this hypothesis. In the absence of isoproterenol, cycloheximide does not cause an increase in β_2 -AR mRNA as it does β_1 -AR mRNA. In the presence of isoproterenol, however, both β_1 - and β_2 -AR mRNA pools are increased dramatically. Since c-fos and junB are induced very rapidly in C_6 glioma cells, these transcription factors are prime candidates for the repression of β_2 -AR mRNA following isoproterenol stimulation. Our data also suggest a role for protein synthesis in the down-regulation of β_1 -AR mRNA. An experiment in which cycloheximide was added at various times to cells stimulated by isoproterenol indicated that, while β_2 -AR mRNA down-regulation could not be blocked by addition of cycloheximide 4.5 minutes or more after the addition of isoproterenol, β_1 -AR mRNA down-regulation could be blocked by addition of cycloheximide as late as 40 minutes after the onset of stimulation. Thus, it is clear that there is a fundamental difference in the nature of the down-regulation of β_1 - and β_2 -AR mRNA species induced by isoproterenol stimulation.

We have further isolated the effects of culture conditions on the response of C_6 glioma cells to various treatments. In an experiment where the same number of cells were plated on dishes of various sizes and grown for 2 days, it became clear that cell density is an important variable in mRNA regulation studies. In cells grown at a moderate density, both β_1 - and β_2 -AR mRNA were transiently up-regulated by isoproterenol stimulation, whereas in very high or low density cells the mRNAs of both subtypes were simply down-regulated. This finding answers a long standing question raised by data reported by two other groups working in the field: how can β_2 -AR mRNA be transiently up-regulated by agonist stimulation in cell suspension and only down-regulated in those same cells in monolayer? The importance of cell density was also demonstrated in the expression of the gap junction protein,

connexin43. Connexin43 and is induced to higher expression by cell-cell contact and by isoproterenol. The response to isoproterenol, however, was highly cell density dependant. Cells of high density responded to isoproterenol induction of connexin43 very poorly, if at all, whereas low density cells responded well: a 3- to 4-fold induction of connexin43 mRNA after 4 hours of stimulation.

We have continued our study of crosstalk between the serotonergic and β -adrenergic receptor systems. Stimulation of C_6 glioma cells with serotonin itself results in a small but reproducible transient up-regulation and eventual down-regulation of β -AR mRNA. This response is also observed when the cells are treated with putative 5HT₂ antagonist, ketanserin. We are presently in the process of determining the primary receptor subtype involved in C_6 glioma cells. We are also examining the effects of β -AR agonist and antagonist on the expression of the 5HT₂ receptor.

We have initiated a study of the possible involvement of the peripheral benzodiazepine receptor, pBzR, in carbamazepine's effects on β -AR mRNA regulation. In this study, we have obtained preliminary evidence that pBzR ligands, PK 11195 and RO 5-4864, can up-regulate β_2 -AR mRNA after a 3 day treatment. The two drugs together act synergistically, suggesting the role of the pBzR in neurosteroid biosynthesis as the mediator of this effect. Since these drugs can dramatically alter the number of mitochondria in C_6 cells, the effects of these drugs could be very general.

II. Significance to Mental Health Research and the Program of the Institute.

Understanding the basis of β -AR mRNA regulation is essential to an understanding of the regulation of β -adrenergic receptors on cell surfaces. The majority of β -AR in the brain are glial. Since glia play an important role in neural modulation and maintenance, the study of β -AR regulation in C_6 glioma cells could lead to important findings that relate to mental disease.

For example, both β -AR and serotonergic receptors have been implicated in depression. Drugs that are effective in treating depression are known to block reuptake of norepinephrine or serotonin or affect β -AR or serotonergic function or number. A link between the expression of these two types of receptors may explain why both are implicated in depression. An old theory of depression suggests that lack of serotonergic tone in the brain is the etiology of depression, and the possible depression of β -AR expression by serotonin stimulation may give substance to this hypothesis.

III. Proposed Course of Project

We plan to further characterize the interaction between β -AR and serotonergic receptors at the level of mRNA expression. What are the subtypes of serotonin and β -adrenergic receptors involved? Does the interaction go both ways? Are G-proteins also affected? In a study just begun in collaboration with Dr. Peter Lesch, we will be examining this question.

We also plan to investigate the role of the peripheral benzodiazepine receptor in β -AR mRNA regulation. The possibility that neurosteroid biosynthesis may be stimulated by carbamazepine could open an important avenue for the study of the role of neurosteroids in the regulation of receptors.

We plan to continue to probe the basic mechanism of mRNA regulation of β -AR from both points of control: transcription and translation. Of interest is how glial cells regulate mRNA expression via contact with other cells or cytoskeletal differentiation (as typified by connexin43 expression and gap junction formation).

Finally, we also plan to study the effect of isoproterenol stimulation on ion channel fluxes in C₆ glioma cells by electrophysiological measurements.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02298-07 BP

PERIOD COVERED
October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Receptor Regulation in Cultured Cerebellum Granule Cells

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Fumihiko Fukamauchi, M.D., Ph.D.	Visiting Fellow	BPB, NIMH
Jotaro Akiyoshi, M.D., Ph.D.	Visiting Fellow	BPB, NIMH
Christopher Hough, Ph.D.	Chemist	BPB, NIMH
Peter Leeds	Biologist	BPB, NIMH
D.-M. Chuang, Ph.D., Chief, Unit on Molecular Neurobiology, BPB, NIMH		

COOPERATING UNITS (if any)

LAB/BRANCH
Biological Psychiatry Branch

SECTION
Unit on Molecular Neurobiology

INSTITUTE AND LOCATION
NIMH, Bethesda, MD 20892

TOTAL STAFF YEARS:
1.4

PROFESSIONAL:
1.4

OTHER:
0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We have studied factors involved in the regulation of phospholipase C-coupled receptors in cerebellar granule cells. In response to stimulation with the muscarinic receptor agonist carbachol, both m_3 - and m_2 -muscarinic receptor mRNA are down-regulated but with a distinct time-course. Exposure of cells to subtype-selective and nonselective antagonists induce differential up-regulation of m_2 - and m_3 -receptor mRNA. The agonist and antagonist-induced effects are associated with a respective down- and up-regulation of the muscarinic receptor transcription rate. m_3 -receptor mRNA and c-fos mRNA are also up-regulated by endothelin, a novel neuropeptide. Disruption of microtubules by colchicine results in a loss of muscarinic receptor number, m_3 -receptor mRNA, and carbachol-induced phosphoinositide turnover, while m_2 -receptor mRNA is markedly up-regulated.

The effects of 5-HT₂ receptor agonists (5-HT, DOI) and antagonists (mianserin, ketanserin) on 5-HT₂ receptor mRNA expression have also been in cerebellar granule cells. Pretreatment with 5-HT or DOI induced a rapid desensitization of 5-HT₂ receptor-mediated PI response and a subsequent increase of ³H-ketanserin binding to 5-HT₂ receptors. The increase in 5-HT₂ receptor binding was associated with an increase in both the B_{max} and K_d value of ³H-ketanserin binding. Moreover, the up-regulation was temporally correlated with an increase of 5-HT₂ receptor mRNA level (from 1-24 hours after treatment). Conversely, preexposure to the 5-HT₂ antagonists mianserin and ketanserin induced a time-dependent decrease of 5-HT-induced response but a concurrent loss of 5-HT₂ receptor binding sites. Mianserin-induced 5-HT₂ receptor down-regulation was accompanied by a marked loss of 5-HT₂ receptor mRNA. Thus, in cerebellar granule cells, the number of 5-HT₂ receptors and 5-HT₂ receptor mRNA levels are regulated by 5-HT₂ receptor agonists and antagonists in an unusual manner.

I. Project Description

Objectives, Methods Employed, and Major Findings

Cerebellar granule cells are prepared from neonatal rats to allow differentiation in culture into glutamatergic neurons with a purity of greater than 90%. We have shown that these neurons possess a variety of neurotransmitter receptors coupled to phospholipase C and other effector systems, thus providing an excellent model system to study drug-induced alteration in the activity and expression of neurotransmitter receptors as well as cross-talk between distinct classes of receptors. Our goal is to obtain a better understanding of the regulation of receptor signal transduction mechanisms and thereby to gain insights into the etiology and treatment of mental illness.

Cultured cerebellar granule cells express in culture mRNA for muscarinic m_3 - (positively coupled to phospholipase C) and m_2 - (negatively coupled to adenylate cyclase) receptors. In response to stimulation with their common receptor agonist carbachol, both m_3 - and m_2 -muscarinic receptor mRNA are down-regulated; however, these two events can be temporally dissociated. Carbachol-induced down-regulation of m_3 -muscarinic receptor mRNA is blocked by its selective receptor antagonist pirenzepine and 4-Diphenyl- acetoxy-N-methyl piperidine (4-DAMP) and the nonselective antagonist atropine, while the down-regulation of m_2 -mRNA is inhibited by its selective blocker AF-DX 116 and atropine. Conversely, persistent exposure of these neurons to atropine results in significant increase in the level of m_3 - and m_2 -receptor mRNA but with a discrete time course. Nuclei RNA runoff experiments have demonstrated that the transcription rate of m_3 -muscarinic receptor mRNA is markedly up-regulated by atropine, but down-regulated by carbachol. Moreover, the stability of m_3 -mRNA is enhanced by either atropine or carbachol treatment. Subtype-specific antagonists, 4-DAMP and AF-DX 116 also cause up-regulation of the mRNA for their respective receptor subtype. Since these cerebellar neurons are not innervated by acetylcholine in culture, these results raise the interesting possibility that muscarinic receptor antagonists have the intrinsic ability to up-regulate their respective receptor mRNA. The up- and down-regulation of muscarinic receptor mRNA may have physiological and pathological significance as abnormality of muscarinic neurotransmission has been implicated in memory and cognitive dysfunction, Alzheimer's disease, motor dysfunction, and side effects of antipsychotic drugs.

Cytoskeleton has been implicated in the pathogenesis of Alzheimer's disease and is a possible site of tricyclic antidepressant drugs. Moreover, cytoskeleton may be a candidate for transducing the signal from the plasma membrane to the nucleus. We have employed a disrupter of microtubule structure, colchicine, as a tool to study its influence on the level of muscarinic and β -adrenergic receptor mRNA. In cerebellar granule cells, we have found that disruption of microtubules by colchicine produces a time- and dose-dependent decrease in m_3 -receptor density and mRNA level. This effect is reversed by taxol, a microtubule stabilizing agent. Moreover, down-regulation of m_3 -mRNA induced by carbachol overlaps with that induced by colchicine and is partially reversed by taxol, suggesting a role of microtubules in the agonist-induced decrease of m_3 -mRNA content. Our more recent data indicate that colchicine causes an opposite effect on m_2 -receptor mRNA levels -- an increase in m_2 -mRNA content that is reversed by taxol. We have observed similar differential effects on β_1 - and β_2 -adrenergic receptor mRNA in C_6

glioma cells. Colchicine induces a time-dependent up-regulation of β_2 -receptor mRNA but down-regulation of β_1 -receptor mRNA. Our results underline the complexity of the regulation of muscarinic and β -adrenergic receptor subtypes and the role of the cytoskeleton in cell-surface receptor gene expression.

The effects of endothelin, a novel neuropeptide, on the expression of mRNA for m_3 -muscarinic cholinergic receptors and c-fos have been explored in cerebellar granule cells. It was found that endothelin elicits a relatively rapid increase in m_3 -muscarinic receptor mRNA with a peak effect at 2 hrs. This effect is preceded by an increase of c-fos mRNA and is abolished when c-fos mRNA induction is prevented by 2-aminopurine or cycloheximide. Suppression of endothelin-1-induced phosphoinositide breakdown by a brief pre-exposure to phorbol ester, which activates protein kinase C, also markedly inhibits the increase of mAChR mRNAs. This finding suggests that this novel neuropeptide is a positive transcriptional regulator acting through the inositol trisphosphate/diacylglycerol signalling pathway.

In order to expand our understanding of the control of neurotransmitter receptor gene expression, we have looked into the effects of 5-HT receptor agonists, antagonists, and psychotropic drugs. It has become clear that 5-HT, by interacting with multiple 5-HT receptors, induces an array of neurophysiological responses including sleep, cognition, mood control, and sexual behaviors. We have demonstrated that cerebellar granule cells express 5-HT₂ receptor subtypes coupled to phospholipase C through a pertussis toxin-sensitive mechanism. Prestimulation of these neurons with 5-HT or DOI (2,5-dimethoxy-4-iodophenylisopropylamine), a putative 5-HT₂ receptor agonist, results in a time-dependent desensitization of 5-HT-induced phosphoinositide response. Interestingly, ³H-ketanserin binding to 5-HT₂ receptors is increased following the onset of desensitization induced by 5-HT or DOI. This augmentation persists for at least 24 hours and is associated with an increase in the B_{max} and K_d of ³H-ketanserin binding to 5-HT₂ receptors. The amount of 5-HT₂ receptor mRNA detected by Northern blot hybridization is increased by 60-100% in parallel with the up-regulation of 5-HT₂ receptor binding sites induced by 5-HT or DOI, while the alpha subunits of Gi and Go proteins are found to be unchanged.

In a related series of experiments, we examined the effects of two 5-HT₂ receptor antagonists, mianserin and ketanserin, on 5-HT₂ receptor-mediated signal transduction. Both antagonists elicit a time-dependent desensitization of 5-HT-induced phosphoinositide turnover and a concomitant decrease of ³H-ketanserin binding to 5-HT₂ receptors. These effects are fully manifest about two hours after exposure with a partial reversal thereafter. Preliminary results show that mianserin-induced desensitization and receptor down-regulation are accompanied by a decrease of the 5-HT₂ receptor mRNA levels. This effect might be clinically relevant, as mianserin is an antidepressant which has been shown to induce a similar down-regulation of 5-HT₂ receptor binding sites and their effector response in vivo. Results also demonstrate, for the first time, that the expression of 5-HT₂ receptors is regulated by their agonists and antagonists in an unusual manner, namely, up-regulation by agonists and down-regulation by antagonists.

II. Significance to Mental Health Research and Program of the Institute

The present studies have provided the molecular basis involved in the homologous desensitization of phospholipase C-coupled receptors, notably muscarinic and 5-HT₂ receptors. Certain mental illnesses, such as Alzheimer's disease, seizures, mania, depression, and side effects of psychoactive drugs are likely to be associated with abnormalities of this receptor-mediated response and changes in the structure of cytoskeletons. Modulation of the activity and expression of neurotransmitter receptors may underline the action of certain psychotropic drugs. The knowledge we have obtained will unequivocally advance our understanding of the biochemical mechanisms of this receptor-mediated effector system and the molecular basis of the action of psychotropic drugs and pathogenesis of certain mental illnesses.

III. Proposed Course of Project

(1) To explore the promoter regulation of the expression of muscarinic and 5-HT₂ receptors; (2) to investigate possible cross-regulation between m₂- and m₃-muscarinic receptors and 5-HT_{1A} and 5-HT₂ receptors in cerebellar granule cells; and, (3) to examine the in vivo effects of long-term pharmacological manipulations of the expression of 5-HT receptor subtypes in the CNS.

Publications

Akiyoshi J, Hough C, Chuang D-M. Regulation of 5-HT₂ receptor mRNA levels of 5-HT₂ receptor agonists and antagonists in cultured cerebellar neurons. Neurosci Abstracts 1992, in press.

Fukamauchi F, Hough C, Chuang D-M. m₂- and m₃-Muscarinic receptor mRNAs have different responses to microtubule-affecting drugs. Mol Cell Neurosci 1991;2:315-319.

Fukamauchi F, Hough C, Chuang D-M. Role of microtubule structure in the maintenance of m₃-muscarinic acetylcholine receptor mRNA levels, Mol Cell Neurosci 1991; 2:123-129.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02467-04 BP
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Receptors for Endothelin and Sarafotoxin in Neurons		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Wan-Wan Lin, Ph.D. Assoc. Professor, National Taiwan University, Taipei, Taiwan De-Maw Chuang, Ph.D., Unit Chief, BPB, NIMH J. Kiang, Ph.D., Walter Reed Army Hospital		
COOPERATING UNITS (if any) Dept of Pharmacology, College of Medicine, National Taiwan University, Taipei, Taiwan; Walter Reed Army Hospital, Washington D.C.		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, Bethesda, MD		
TOTAL STAFF YEARS: 1.2	PROFESSIONAL: 1.2	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have conducted pioneering studies demonstrating that <u>endothelin-1 (ET)</u> is a novel neuropeptide in the CNS. We have found that <u>cerebellar granule cells</u> not only synthesize and release ET upon stimulation, but also express ET-specific receptors coupled to <u>phospholipase C</u> . Stimulation of cerebellar neurons also leads to release of preloaded <u>D-aspartate</u> . Both ET-induced activities are Ca^{2+} dependent and Na^+ independent, but the release process requires ET-induced phosphoinositide hydrolysis and other receptor-mediated events. Because increasing evidence indicates that glial cells are a target for endothelin, we have characterized the effector responses mediated by ET receptors in <u>C₆ glioma</u> cells. Stimulation with ET induces phosphoinositide hydrolysis to generate inositol trisphosphate and causes <u>Ca^{2+} influx</u> through a receptor-gated channel. Both events are dependent on external Ca^{2+} and sensitive to <u>inorganic Ca^{2+} channel blockers</u> (Cd^{2+} , La^{3+} , and Mn^{2+}). The former response is mediated by a pertussis toxin-sensitive G protein, leading to intracellular Ca^{2+} mobilization, while the latter is positively regulated by protein kinase C and results in further Ca^{2+} increase to sustain phosphoinositide turnover. The presence of <u>Ca^{2+} ionophores</u> A23187 and ionomycin also potentiates the phosphoinositide response to ET and <u>ATP</u> . Conversely, KCl in the range of 15 to 55 mM markedly inhibits ET and ATP-induced phosphoinositide breakdown and attenuates the intracellular Ca^{2+} increase elicited by these two agonists. These results demonstrate that the phosphoinositide response mediated by these two types of receptors are tightly controlled by intracellular Ca^{2+} levels. Based on the selectivity of adenine nucleotides, it can be concluded that <u>P_{2y} purinoceptors</u> are expressed in <u>C₆ glioma</u> cells. As in ET receptors, <u>purinoceptors</u> in glioma cells are coupled to both phospholipase C and Ca^{2+} influx and show homologous desensitization via a protein kinase C-independent mechanism.		

I. Project Description

A. Objectives, Methods Employed and Major Findings

Endothelin-1 (ET) stimulated ^3H -inositol phosphate formation by 6-8 fold in cultured cerebellar granule cells prelabeled with ^3H -myo-inositol (with an EC_{50} of about 1 nM). The effect was sodium-independent but calcium-dependent; however, the calcium dependency was unaffected by 1 mM Co^{2+} , Mn^{2+} and amiloride and 10 μM dihydropyridine derivatives. Prestimulation of granule cells with ET-1 for as little as 30 seconds induced a virtually complete desensitization of phosphoinositide (PI) response to further stimulation with ET-1 and sarafotoxin S6b. This desensitization was homologous as PI responses to 5-HT, NE, histamine, carbachol and glutamate were unaffected, even when the prestimulation time was prolonged to 24 hours. ET-1 and its homologues also induced a robust increase of PI breakdown in primary cultures of cerebellar astrocytes and C_6 -glioma cells with a rank order of potency of $\text{ET-1} \geq \text{ET-2} \geq \text{S6b} > \text{ET-3}$. Short-term (15 min) pretreatment with phorbol ester attenuated ET-induced inositol phosphate formation in all three cell types. However, long-term (24 hr) phorbol ester treatment attenuated PI response to ET induced responses in granule and glioma cells but enhanced the same response in astrocytes. Long term treatment with pertussis toxin attenuated ET-induced responses in astrocytes and glioma but had no effect on granule cells, indicating that distinct G-proteins are involved in the coupling of ET receptors to phospholipase C.

In an attempt to elucidate the neurophysiological role of ET receptors in cerebellar granule cells, we have demonstrated that ET stimulated the release of preloaded ^3H -D-aspartate, a marker for endogenous glutamate. Although this ET effect was also calcium-dependent and insensitive to Co^{2+} (1 mM) and dihydropyridines (1 μM), the response was enhanced by activation of protein kinase C and inhibited by kinase C depletion. These observations suggest a role of ET-induced PI turnover and $[\text{Ca}^{2+}]_i$ increase in the release of the neurotransmitter. Our preliminary results also indicate that cultured granule cells synthesize and release ET-like materials, further supporting the notion that ET is a neuropeptide in the excitatory neurons of cerebellar granule cells.

In C_6 -glioma cells, ET- and ATP-induced PI turnover was potentiated by calcium ionophores and associated with about 4-fold increase in $[\text{Ca}^{2+}]_i$. The ET-induced $[\text{Ca}^{2+}]_i$ increase was dependent on calcium influx and mobilization but insensitive to calcium channel blockers such as verapamil and dihydropyridines. Cd^{2+} , Mn^{2+} and La^{3+} at 1 mM partially blocked the ET-induced increase of $[\text{Ca}^{2+}]_i$. Depolarizing concentrations of potassium inhibited ET- and ATP-induced PI turnover and the increase of $[\text{Ca}^{2+}]_i$. While 3-hr treatment with sphingosine, staurosporine and PTX was without effect, the ET-induced $[\text{Ca}^{2+}]_i$ increase was enhanced by 10 min treatment with a phorbol ester but inhibited by 24 hr pretreatment. Prestimulation of glioma with ET desensitized the $[\text{Ca}^{2+}]_i$ increase induced by ET, but not that induced by ATP, indicating a homologous desensitization.

The ATP-induced PI turnover and $[\text{Ca}^{2+}]_i$ increase have been further characterized in C_6 glioma cells. The receptor preference for various adenine nucleotides indicates that P_{2y} purinoceptors are expressed in C_6 glioma. ATP-stimulated PI metabolism was partially dependent on extracellular Ca^{2+} and Na^+ . In Ca^{2+} -free medium, ATP caused only a transient increase in $[\text{Ca}^{2+}]_i$ as opposed to a sustained

$[Ca^{2+}]_i$ increase in normal medium. The ATP-induced elevation of $[Ca^{2+}]_i$ was resistant to Na^+ depletion but was attenuated by La^{3+} . The latter failed to affect ATP-induced PI turnover. These results suggest that, similar to ET-specific receptors, purinoceptors in C_6 glioma cells are coupled to phospholipase C and Ca^{2+} influx. Prestimulation of glioma cells with ATP led to homologous desensitization of ATP-induced PI turnover. Unlike phorbol ester-induced heterologous PI desensitization, ATP-induced homologous desensitization did not involve protein kinase C activation.

In a related study to advance our understanding of the regulation of receptors for neuropeptides, we characterized bradykinin receptors in cerebellar astrocytes. Bradykinin induced a robust increase in PI metabolism in a pertussis toxin-insensitive mechanism. Although short-term (≤ 90 min) treatment of cells with a phorbol ester markedly attenuated bradykinin-induced PI response, extended (e.g., 24 hr) pretreatment actually potentiated this response. Similar potentiation was observed by prestimulation of cells with bradykinin, endothelin, or norepinephrine for 24 hours. Our results indicate that cerebellar astrocytes contain phospholipase C-coupled bradykinin receptors which are tightly regulated by protein kinase C in a negative manner.

II. Significance to Mental Health Research and Program of the Institute

The present study has demonstrated that ET elucidates a robust increase in PI metabolism in cerebellar neurons, astrocytes, and C_6 glioma cells. Since receptor-mediated PI hydrolysis is involved in a spectrum of neurophysiological events, including neurotransmission, release of transmitter and LTP, it is anticipated that important roles of ET-1 and its receptors in the CNS will be unraveled rapidly. In fact, a possible role of ET in the pathogenesis of schizophrenia has been suggested. One of the roles of ET receptor in cerebellar granule cells appears to be to trigger the release of the endogenous transmitter glutamate and the expression of certain proteins with a major function. It is also likely that ET receptors in astrocytes have some function. In fact, it was recently reported by a number of laboratories that ET enhances DNA synthesis and proliferation of glial cells. ET-induced calcium influx through receptor-gated channels may also have longer-term effects on cellular activity.

III. Proposed Future Studies

Our proposed future studies are: 1) to elucidate molecular mechanisms involved in the signal transduction of ET receptors in neurons and glial cells, and, 2) to conduct electrophysiological studies on Ca^{2+} channels gated by ET and purinoceptors in neurons and glia.

Publications

Chuang D-M, Lin W-W, Lee CY. Endothelin-induced activation of phosphoinositide turnover, calcium mobilization and transmitter release in cultured neurons and neurally related cell types, J Cardiovasc Pharmacol 1991;17[7]:S85-S88.

Lin W-W, Chuang D-M. Potentiation by Ca^{2+} ionophores and inhibition by extracellular KCl of endothelin-induced phosphoinositide turnover in C_6 glioma cells. *Neurochem Int* (in press).

Lin W-W, Chuang D-M. Regulation of bradykinin-induced phosphoinositide turnover in cultured cerebellar astrocytes: possible role of protein kinase C. *Neurochem Int* (in press).

Lin W-W, Lee CY, Chuang, D-M. Endothelin- and sarafotoxin-induced phosphoinositide hydrolysis in cultured cerebellar granule cells: biochemical and pharmacological characterization, *J Pharmacol Exp Ther* 1991;259:1053-1061.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02468-05 BP

PERIOD COVERED

October 1, 1991 - September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanisms of Action of Psychoactive Drugs

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Xiao-Ming Gao, M.D.	Visiting Fellow	BPB, NIMH
Fumihiko Fukamauchi, M.D., Ph.D.	Visiting Fellow	BPB, NIMH
D.-M. Chuang, Ph.D.	Unit Chief	BPB, NIMH
Reni Li, M.D.	Visiting Fellow	NPB, NIMH
Russell Margolis, M.D.	PRAT Fellow	BPB, NIMH
Robert M. Post, M.D.	Branch Chief	BPB, NIMH
Steven M. Paul, M.D.	Section Chief	NSB, NIMH

COOPERATING UNITS (if any)

Neuropsychiatry Branch, NIMH; Clinical Neuroscience Branch, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Molecular Neurobiology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

2.0

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We have studied several aspects of the action of psychotropic drugs. In cultured cerebellar granule cells, carbamazepine (CBZ) was found to induce a dose-dependent delayed toxicity in a concentration range that overlaps and largely exceeds the therapeutic level. This neurotoxicity may be involved in the side effects and overdosage toxicity of CBZ. Additionally, glutamate induces neurotoxicity of cerebellar granule cells by activation of NMDA receptors. The neurotoxicities induced by CBZ or glutamate are completely blocked by the presence of NMDA. The neuroprotective effect of NMDA is not due to desensitization of NMDA receptors. Carbamazepine-induced neurotoxicity may be due to allosteric interaction of this drug with certain specific domains of NMDA receptors. Another psychotropic drug, lithium, at clinically relevant concentrations (≤ 2 mM) was found to be neurotrophic for cerebellar granule cells. Following a 7-day treatment, the outgrowth of neurites as well as the expression of mRNAs for m₂-muscarinic receptors and c-fos were markedly enhanced. At higher concentrations (≥ 5 mM), this drug elicited a deterioration of neuronal morphology due to lithium-induced neurotoxicity. In a collaborative effort, we found that in rats chronically treated with haloperidol, the phosphoinositide hydrolysis mediated by several neurotransmitter receptor systems were down-regulated, resembling the known effects of lithium. Moreover, combined treatment with nicotine abolished haloperidol's effect on carbachol-induced phosphoinositide turnover in the cortex and hippocampus, but potentiated the same response in the striatum. We also examined the effects of long-term treatment of rats with lithium and CBZ on the mRNA level for connexin43, a major gap junction protein. Lithium but not CBZ tends to reduce connexin43 mRNA levels in most brain regions; however, in the hippocampus, CBZ but not lithium has this effect.

I. Project Description

A. Objectives

We have been studying the regulation of neurotransmitter receptor expression, neurotransmitter-induced production of second- and third-messengers, neurotoxicity, and neuroprotection. We are particularly interested in how each of these phenomena relate to psychotropic drugs' action, especially carbamazepine (CBZ) and lithium. Both in vitro systems using systems using rat cerebellar granule cells and in vivo systems, primarily chronic administration of the neuroleptic haloperidol and psychotropic drugs lithium and CBZ, has been employed in these studies.

B. Methods Employed

Cerebellar granule cells are prepared from eight-day old rats and cultured in vitro for studying the long-term effects of psychotropic drugs and excitotoxins. These cells differentiate in culture into glutamatergic neurons. For in vivo studies, rats were either injected with haloperidol for six weeks or administered a lithium or CBZ diet for one month. Various brain regions were dissected for studying receptor-mediated phosphoinositide hydrolysis or for analysis of connexin43 mRNA, which encodes a gap junction protein controlling intercellular communications.

C. Major Findings

Exposure of cerebellar granule cells to relatively high concentrations of CBZ (30-100 mM) for more than three days induced a decrease in receptor agonist (carbachol, NE, 5-HT and batrachotoxin)-induced phosphoinositide turnover. The loss of these phosphoinositide responses was tightly correlated with a decrease of ³H-ouabain binding to Na⁺, K⁺-ATPase in intact cells (which is a valuable index for quantifying neuronal viability) and a deterioration of cell morphology, indicating that neuronal death had occurred under this treatment regimen. The IC₅₀ value of CBZ was about 45 μM and maximal loss occurred at 100 μM. Interestingly, the CBZ-induced neurotoxicity was completely blocked by the presence of N-methyl-D-aspartate (NMDA) during treatment and this protective effect was blocked by NMDA antagonists, APV and MK-801.

In order to gain insight into the mechanisms of neurotoxicity and neuroprotection, we have collaborated with Dr. Steven M. Paul (Section on Molecular Pharmacology, NSB) to demonstrate that glutamate induced delayed toxicity via activation of NMDA receptors. As with the effect induced by CBZ, the glutamate-elicited neuronal death was also blocked by NMDA. However, a subtle difference existed in the NMDA protection against these two types of excitotoxins. The glutamate toxicity was blocked only when cells were pretreated with NMDA, while NMDA was effective against CBZ toxicity even when NMDA was added 24 hours after CBZ treatment. Although the mechanism(s) underlying NMDA-dependent protection is unknown, we have obtained evidence that argues against the involvement of NMDA receptor desensitization. Thus, following NMDA pretreatment, NMDA receptor-mediated phosphoinositide turnover and [³H]-MK801 binding to NMDA receptors were unchanged. Moreover, the neuroprotection was evident in cells that were pretreated with NMDA followed by drug washout. The neuroprotective effect of NMDA was also independent of the stage of cerebellar granule cell differentiation.

We have also investigated long-term effects of lithium, particularly on m_3 -muscarinic receptors in cerebellar granule cells. At a therapeutically relevant concentration range (0.5-2.0 mM), after three to five days, lithium chloride treatment induced an 80-100% increase in m_3 -mAChR mRNA, but a decrease in m_2 -mAChR mRNA in these neurons. This effect was associated with a slight but significant increase in total muscarinic receptor binding sites and carbachol-induced phosphoinositide hydrolysis. C-fos mRNA and the outgrowth of neurites were also markedly enhanced, indicating a trophic action of lithium. At higher concentrations (≥ 5 mM), this drug induced a drastic reduction of m_2 - and m_3 -mRNA, c-fos mRNA, and a deterioration of neuronal morphology due to induction of neurotoxicity. The neurotoxicity of lithium was not prevented by known neuroprotective/neurotrophic agents such as NMDA and tetrahydroaminoacridine or by the presence of excess myo-inositol.

We collaborated with Dr. Rena Li and her co-workers to study chronic haloperidol effect on phosphoinositide responses to receptor agonists and found that continuous treatment with haloperidol decanoate for six weeks significantly attenuated phosphoinositide metabolism stimulated by dopamine and carbachol in striatal and cortical slices. A significant decrease in NE-sensitive PI turnover was also observed in the frontal cortex, but not in the striatum. Interestingly, chronic combined treatment with nicotine abolished haloperidol's effect on carbachol-induced phosphoinositide metabolism in the frontal cortex and hippocampus, but potentiated the same response in the striatum.

We have also studied the long-term in vivo effect of lithium and CBZ on the expression of the mRNA for connexin43, which encodes a gap junction protein to control the communications between glia and among glia and neurons. Preliminary results suggest that lithium, but not CBZ, tended to reduce connexin43 mRNA levels in various brain regions, but in the hippocampus CBZ, but not lithium, had this effect. Since the β -adrenergic receptor agonist isoproterenol increased connexin-43 mRNA by a cAMP-dependent mechanism in C₆ glioma cells, the in vivo effects of long-term lithium and CBZ could be related to their ability to decrease cAMP.

II. Significance to Biomedical Research and Program of the Institute

A. The neurotoxicity induced by carbamazepine occurs at concentrations that overlap but largely exceed the therapeutic level. The toxicity could, therefore, be related to the side effects of carbamazepine; i.e., teratogenesis and overdosage effects such as abnormal movements, coma, and seizures. The protective effects of NMDA against CBZ toxicity may provide a new avenue for development of a drug that would alleviate the toxicity of CBZ, thereby increasing its clinical efficacy/toxicity ratio.

B. The neurotrophic effects of lithium on cerebellar granule cells occur at a clinically relevant concentration range (≤ 2 mM) and require long-term treatment, suggesting that the trophic action may be pertinent to its therapeutic effects. It might be hypothesized that bipolar depressive illness is the result of a deficiency in the expression of key substances, such as m_3 -muscarinic cholinergic receptors, and that lithium displays its clinical efficacy by promoting their biosynthesis. The chronic effects of haloperidol in receptor-mediated phosphoinositide turnover suggest that mania could be the consequence of hyperac-

tivity of this receptor signalling pathway, and that neuroleptics alleviate the symptoms by suppressing this receptor-mediated activity.

III. Proposed Course of Project

- 1) To define the mechanisms whereby CBZ and lithium induce neurotoxicity;
- 2) to investigate the molecular details involved in the neuroprotection by NMDA;
- 3) to investigate possible interaction between CBZ and NMDA receptors or peripheral benzodiazepine receptors. The latter requires some electrophysiological studies.

Publications

Chuang D-M, Gao X-M, Paul SM. N-Methyl-D-aspartate exposure blocks glutamate toxicity in cultured cerebellar granule cells. *Mol Pharmacol*, in press.

Gao X-M and Chuang D-M. Carbamazepine induces neurotoxicity by a NMDA-reversible mechanism in cultured cerebellar granule cells. *Neurosci Lett* 1992;135:159-162.

Li R, Wing LL, Kirch DG, Wyatt RJ and Chuang D-M. Effects of chronic nicotine and haloperidol administration on muscarinic receptor-mediated phosphoinositide turnover in rat brain slices. *Psychopharmacology*, in press.

Post RM and Chuang D-M. Mechanism of action of lithium: comparison and contrast with carbamazepine. In: Birch NJ, ed. *Lithium and the cell*. New York: Academic Press, 1991;199-242.

Post RM, Weiss SRB and Chuang D-M. Mechanisms of action of anticonvulsants in affective disorders: comparison with lithium. *J Clin Psychopharmacol* 12 [suppl 1], in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02538-03 BP
PERIOD COVERED October 1, 1991 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A study of Effects of HIV-1 on Neurons and Lymphocytes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) D.-M. Chuang, Ph.D., Chief, Unit on Molecular Neurobiology, BPB, NIMH Xiao-Ming Gao, M.D., Visiting Fellow, BPB, NIMH R. Anand, M.D., FDA L. Vitkovic, NIAID S. Ramakrishnan, University of Minnesota		
COOPERATING UNITS (if any) NIAID, NIH; FDA; University of Minnesota		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, Bethesda, MD		
TOTAL STAFF YEARS: 0.6	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have attempted to elucidate the mechanisms of the neuropathogenesis of <u>HIV-1</u> infection. We have investigated the interaction between HIV-1 and <u>cerebellar granule cells</u> in culture using two HIV isolates from <u>AIDS</u> patients with and without overt neurological symptoms. Upon exposure to HIV-1, progressive disruption of neuronal network and destruction of cells occurred in a time- and virus-dose-dependent manner. Immunostaining revealed that <u>only neurons</u>, not astrocytes or other proliferating cells, <u>are killed</u>. Viral proteins and HIV-1-specific DNA and RNA were detected in the culture of cerebellar neurons. HIV-1 isolates from the brain of an AIDS dementia patient is consistently more potent than other isolates from patients with immunological disease. This is the first evidence that cultured neuron is a direct target for HIV-1. In addition, we have preliminary results showing that three <u>protein species isolated from various plants</u> have anti-HIV-1 activity when tested in <u>human peripheral blood lymphocyte systems</u>. </p> <p> This project is temporarily inactive because of the departure of Dr. Anand for an administrative post. We are seeking a new collaborator to continue this study. </p> <p> THIS PROJECT HAS BEEN TERMINATED </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 MH 02539-02 BP
PERIOD COVERED October 1, 1992 - September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Receptor Regulation in Neurohybrid Cell Lines		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
De-Maw Chuang, Ph.D., Unit Chief		BPB, NIMH
Xiao-Ming Gao, M.D., Visiting Associate		BPB, NIMH
Stephen Gucker, Ph.D., NRC Fellow		BPB, NIMH
Carmine Coscia, Ph.D.		St. Louis University
COOPERATING UNITS (if any)		
Department of Biochemistry, St. Louis University, St. Louis, MO		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, Bethesda, MD		
TOTAL STAFF YEARS: 1.2	PROFESSIONAL: 1.2	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have explored molecular details involved in the up-regulation of <u>δ opioid receptors</u> in hybrid neuroblastoma <u>NG 108-15</u> cells treated with the opioid antagonist <u>naltrexone</u> for two days. The up-regulation is associated with an increase in ^3H -DADLE and ^3H -diprenorphine B_{max} values in both light and heavy membrane fractions derived from subcellular fractionations. In contrast, a 5-min exposure to the opioid antagonist naltrexone or ICI 174864 induces a transient <u>down-regulation</u> of δ -opioid receptors prior to up-regulation. Naltrexone and δ -specific antagonists <u>ICI 174864</u> and <u>naltrindole</u> also diminish specific activities of the <u>lysosomal enzymes</u> β -glucuronidase and β -hexoseaminidase. Pretreatment of cells with <u>concanavalin A</u> blocks both the down-regulation and alterations in the lysosomal enzyme activities, suggesting that the initial process of up-regulation of the δ -opioid receptors by antagonists entails down-regulation that may involve lysosomal enzyme activity changes. We also have identified novel intracellular δ -opioid binding sites associated with the nuclei of the same cell line. These <u>nuclear δ-opioid sites</u> have been determined by <u>immunohistochemical studies</u> on cryostat sections with an anti-opioid receptor antibody and by the K_d and B_{max} values of the binding of ^3H -diprenorphine to the highly purified nuclear preparation. Opioid binding sites have also been shown in subnuclear preparations. Agonists ^3H -DADLE and ^3H -DSLET bind with high affinity to <u>nuclear membranes</u> and with lower affinity to chromatin. In contrast, the partial agonist ^3H -diprenorphine high affinity binding sites are predominant in <u>chromatin</u> , while low affinity sites are in the nuclear membrane. Accordingly, <u>GppNHP sensitivity</u> of ^3H -DADLE binding is detected in nuclear membranes but not in chromatin. Both agonist and partial agonist binding sites in nuclear membrane and chromatin are abolished by treatment of cells with cycloheximide. Taken together the results suggest that NG 108-15 cells contain newly synthesized G protein-coupled δ -opioid receptors in nuclear membrane and internalized, uncoupled opioid binding sites in chromatin.		

I. Project Description

A. Objectives

The aim of this study is to employ neuroblastoma cell lines as a model to investigate mechanisms underlying δ opioid antagonist-induced up-regulation of this receptor site and to identify novel intracellular δ opioid receptors in an attempt to expand our understanding of drug tolerance and dependence. We also used a neuroblastoma cell line to explore the role of G protein in the down-regulation of G protein-coupled receptors (notably muscarinic receptors) induced by the receptor agonists.

B. Methods Employed

Two related neurohybrid cell lines, NG 108-15 (neuroblastoma X glioma) and NCB-20 (neuroblastoma X fetal brain cell) were used in this study. Subcellular fractionation of cell homogenates obtained by a cell cracker was performed using sorbitol gradient centrifugation. Receptor binding assays were conducted using the conventional methods. Nuclei were isolated by centrifugation through a 1.7 M sucrose cushion. Immunohistochemical localization of δ opioid receptors was studied using anti-idiotypic anti-opioid receptor antibody in conjunction with a cryostatic section of cells examined.

C. Findings

According to current concepts, agonists can effect the down-regulation of cell surface receptors while antagonists can cause their up-regulation. We have discovered that opioid antagonists naltrexone and ICI 174864 induce a transient down-regulation of δ opioid receptors prior to up-regulation in NG 108-15 cells. The loss in cellular receptors was time- and antagonist concentration-dependent but could not be induced by the highly μ -selective opioid antagonist CTAP. Muscarinic binding was neither affected by short- nor long-term exposure of the cells to naltrexone. In the same neurohybrid cells the opioid agonist [D-Ala², D-Leu⁵] enkephalin (0.1 μ M, 60 min) down-regulated δ opioid receptors to a comparable extent. Similar changes in opioid binding of subcellular fractions were elicited with [D-Ala², D-Leu⁵] enkephalin and naltrexone. Namely, there is a decrease in the heavy membrane population of receptors and an increase in light membrane sites. Since heavy membranes are enriched in plasma membrane receptors while light membranes contain intracellular sites, these findings indicate that internalization is occurring in both instances. Naltrexone and the δ -specific antagonists ICI 174864 and naltrindole also diminished specific activities of the lysosomal enzymes, β -glucuronidase and β -hexoseaminidase. In contrast, opioid agonist-induced down-regulation was accompanied by a 21-23% increase in the specific activities of both enzymes measured in cell homogenates. Pretreatment of cell cultures with concanavalin A (0.25 mg/ml) blocked both the down-regulation and the alterations in the lysosomal enzyme activities elicited by agonist and antagonist, suggesting that the latter is an opioid receptor-mediated process. Chronic Bt₂CAMP treatment of neurohybrid cells, which like agonists cause opioid receptor down-regulation, also elevated lysosomal enzyme activities. Antagonist-induced down-regulation of δ opioid receptors could also be demonstrated *in vivo*. Administration of naltrexone (ip, 10 mg/kg) to rats resulted in lowered

^3H -[D-Ser², L-Leu⁵] enkephalyl-Thr B_{max} values in hindbrain, but not in striatum, hippocampus or cortex after 60 min. Longer naltrexone exposure (6-48 h), caused up-regulation of δ opioid binding in all four regions. These data suggest that the initial process of up-regulation of δ opioid receptors by antagonists entails down-regulation that differs mechanistically from that of agonists with respect to lysosomal enzyme activity changes.

Nuclear opioid binding sites have been discovered in NG 108-15 and NCB-20 neurohybrid cell lines. Marker enzyme analyses attested to the high degree of purity of the nuclear preparations. Immunohistochemical studies on cryostat sections of NG 108-15 cells with an antibody to the opioid receptor corroborated a nuclear localization. ^3H -[D-Ser², L-Leu⁵] enkephalyl-Thr (^3H -DSLET), ^3H -[D-ala², D-Leu⁵] enkephalin (^3H -DADLE) and ^3H -diprenorphine binding parameters, K_d and B_{max}, as well as heterologous competition binding and stereospecificity data, satisfied criteria for the presence of δ opioid sites in purified nuclear preparations. Neither μ -[D-ala², mephe⁴, gly-ol⁵] enkephalin (DAMGE), dihydromorphine nor α -[U69593] specific binding was detectable in purified nuclear preparations. Rates of association and dissociation of ^3H -DSLET were comparable to values obtained previously for opioid receptors. Opioid binding was also shown in subnuclear preparations from NG 108-15 cell cultures. Agonists ^3H -DADLE and ^3H -DSLET bind with high affinity to nuclear membranes and with lower affinity to chromatin. In contrast, partial agonist ^3H -diprenorphine high affinity binding sites were predominant in chromatin, while low affinity binding was found in the nuclear membrane. Accordingly 5'-guanylylimidodiphosphate (Gpp(NH)p) sensitivity of ^3H -DADLE binding was detected in nuclear membranes but not in chromatin. Both agonist and partial agonist opioid binding to nuclear membranes and chromatin was abolished upon cycloheximide treatment of NG 108-15 cells. Taken together, the results suggest that NG 108-15 cells contain newly synthesized GTP binding regulatory protein (G protein)-coupled δ opioid receptors in nuclear membranes and internalized, uncoupled opioid binding sites in chromatin.

In a related study, we have used NCB-20 cells to investigate the role of G protein in the desensitization and down-regulation of mAChRs. NCB-20 cells possess both m₁- and m₄-mAChRs which are presumably coupled to the stimulation of phospholipase C and inhibition of adenylate cyclase, respectively. In pilot experiments, we have demonstrated that upon long-term exposure, some muscarinic agonists, such as carbachol, induce mAChR down-regulation, but other agonists such as pilocarpine fail to induce down-regulation. It will be established through the use of digitonin-permeabilized cells and stable GDP and GTP analogs whether this distinction between different agonists is due to differences in their ability to activate G proteins. Second messenger efficacy of different agonists will also be examined for its possible role, if any, in mAChR down-regulation. In addition to direct receptor binding, mRNA levels will be examined using different agonists and under different conditions of G protein activation. Finally, the identity of G proteins possibly involved in receptor down-regulation will be examined by using antisense oligonucleotides directed against specific G protein alpha subunits in order to block or reduce their expression in NCB-20 cells.

II. Significance to Mental Health Research and Program of the Institute

Neurotransmitter receptor-mediated effector-response has been implicated in synaptic transmission, axonal regeneration, and the process of learning and memory. Alteration of this receptor-mediated event has also been suggested to be involved in some pathological states such as Alzheimer's disease, seizures, mania, depression, and alcoholism. In the present study, we have characterized these receptor-mediated responses and studied their regulatory mechanisms. Undoubtedly, the information obtained will advance our understanding of the molecular mechanisms of the etiology of some mental or neurological illnesses and might eventually lead to the discovery of therapeutic modalities that can prevent, alleviate, or cure disease states related to receptor malfunction. The understanding of mechanisms involved in δ opioid receptor up-regulation will provide insight into problems associated with narcotic abuse. The discovery of nuclear δ opioid receptors may have implications for opioid receptor-mediated events that require gene expression, such as opioid tolerance and dependence.

III. Proposed Course of Project

(1) To verify that δ opioid receptors in nuclear membranes are G-protein-coupled newly synthesized receptors, while chromatin-associated δ receptors are uncoupled, internalized receptors. The effects of δ receptor agonists on cAMP production will be examined in these studies.

(2) To investigate whether the differences in the ability of various muscarinic receptor agonists to induce the receptor down-regulation is due to their distinct properties in activating their corresponding G proteins.

(3) To identify G proteins possibly involved in receptor down-regulation by using antisense oligonucleotides directed against specific G-protein alpha subunits.

Publications

Belcheva M, Barg J, Gloeckner C, Gao X-M, Chuang D-M, Coscia CJ. Antagonist-induced transient down-regulation of δ opioid receptors in NG 108-15 cells. *Mol Pharmacol*, in press.

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Belcheva M, Barg J, Rowinski J, Gloeckner CA, Ho A, Gao X-M, Chuang D-M, Coscia CJ. Novel intracellular opioid binding sites associated with the nuclei of neurohybrid cells. *J. Neurosci.*, in press.

Z01 MH 02539-02 BP

Report not available at time of printing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02583 02 CNE
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Melancholia as a Dysregulation of the Stress Response		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Co-PI: Philip W. Gold, M.D., Chief, Clinical Neuroendocrinology Branch, NIMH Co-PI: Mitchel A. Kling, M.D., Chief, Unit on Affective Disorders, Clinical Neuroendocrinology Branch, NIMH Others: Dr. L.S. Brady Senior Staff Fellow CNE, NIMH Dr. G. Cizza Visiting Fellow CNE, NIMH Dr. M.B. DeBellis PRAT Fellow NIMH Dr. L.D. Dorn IRTA Fellow CNE, NIMH Dr. M. Enk Visiting Fellow CNE, NIMH		
(Continued on p. 2)		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Neuroendocrinology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 8	PROFESSIONAL: 6	OTHER: 2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have advanced many lines of evidence indicating that the clinical and biochemical manifestations of <u>melancholic depression</u> reflect a concomitant activation of the <u>corticotropin releasing hormone</u> and locus <u>ceruleus-norepinephrine</u> systems that have escaped their usual counter-regulatory mechanisms. To support this hypothetical model, we have shown that a many classes of antidepressants given chronically promote a decrease in PVN CRH mRNA expression, content, and secretion, including not only <u>tricyclic</u> antidepressants (e.g. imipramine), but also <u>MAO inhibitors</u> (e.g. phenelzine), specific <u>serotonin uptake inhibitors</u> (e.g. fluoxetine), <u>alpha-2 receptor antagonists</u> (e.g. idosoxan), <u>anticonvulsants</u> (e.g. carbamazepine), and <u>GABA agonists</u> (e.g. alprazolam). Clinically, we have shown that both the chronic administration of Moreover, we have shown that these effects are specific to the resting state of the organism; hence, they are most pronounced in those in whom there is a sustained activation of the PVN CRH neuron, present on either a genetic or environmental basis. Our clinical data show that normal <u>aging</u> is associated with a progressive fall in CSF CRH, while our basic data show that aging in the rat is associated with a <u>progressive central adrenal insufficiency</u> due to a decrease in CRH synthesis and release. These data are in contrast with the progressive increase in plasma cortisol levels and CSF CRH levels seen in patients with a history of melancholia and support the theory that progressive episodes of CRH hypersecretion in melancholia predispose to the exacerbating course of recurrent affective illness. Our data also suggest that while acute glucocorticoid secretion serves to restrain the generalized stress-response from overshooting, chronic glucocorticoid secretion may enhance it, and hence, exacerbate the course of melancholic depression. Specifically, glucocorticoid administration both accelerates the rate of electrically-induced limbic kindling and the effects of <u>limbic kindling</u> on the expression of <u>CRH mRNA</u> in <u>hippocampal</u> GABAergic neurons where CRH is normally not expressed. We have also advanced data that experimentally-induced hyperthyroidism is associated with enhanced CRH mRNA expression and content in the PVN, as well as enhanced CRH release from rat hypothalamic organ cultures. These data are of interest in the light of preliminary data from our group regarding central hyperthyroidism in patients with severe melancholic depression, who also show evidence of hypothalamic, CRH-mediated hypercortisolism.		

Objectives: A principal goal of the present project is to elucidate the molecular and biochemical mechanisms of physical and emotional stress and their relevance to major psychiatric disorders, with particular emphasis on melancholic depression. This project represents one of several whose aim is to define the molecular and biochemical bases of diseases that occur as dysregulations of the stress response and to develop more rapid and specific therapeutic interventions based on neuroendocrine pharmacologic modulation of the major effectors of the stress response. A long-term goal is to utilize the information gained regarding pathophysiological features of melancholia to focus on candidate genes whose dysregulation confers susceptibility to this illness. A corollary goal of this project is to compare and contrast pathophysiological mechanisms in the hypercortisolism of major depression and Cushing's disease and to develop improved means for their differential diagnosis. Our work is hypothesis driven, proceeds in parallel on the clinical research unit and in the basic laboratory, and requires the close collaboration and cooperation of neuroendocrinologists, psychiatrists, molecular biologists, neurobiologists, and neuropharmacologists.

Methods employed: A variety of techniques have been developed and/or are applied on the clinical research unit for the clinical study of patients with melancholic depression and controls, including methods for the assessment of basal metabolic rate, norepinephrine spillover rate into arterial plasma, circadian pattern of neurotransmitter and neuropeptide release into cerebrospinal fluid, assessment of the cortisol and ACTH production rates, and many paradigms for the assessment of the functional integrity of each component of the hypothalamic pituitary-adrenal, gonadal, and thyroid axes. In the laboratory, we have raised antisera to a variety of neuropeptides including ovine and rat/human CRH, ACTH and various fragments, beta-endorphin, atrial natriuretic factor, arginine vasopressin, oxytocin, dynorphin, tumor necrosis factor, interleukin 1, interleukin 2, interleukin 6, neuropeptide Y, neuropeptide YY, cholecystokinin, and met-enkephalin. These antisera are used for radioimmunoassay, immunohistochemistry, and immunoneutralization studies. Affinity purification of antibody and immunohistochemistry procedures for these peptides have also been established. We also utilize gel and HPLC chromatographic methods for purification and identification of ovine and rat/human CRH, and for POMC fragments. Specific peptide antagonists for CRH, arginine vasopressin receptor subtypes, oxytocin, and cholecystokinin are also utilized. Additional methodologies include high resolution autoradiography, an ACTH bioassay in which rat adrenal corticosterone is examined as an endpoint, dispersed to anterior pituitary cell cultures for examination of CRH activity or various extrahypothalamic substances with CRH bioactivity but not immunoreactivity, and a hypothalamic organ culture system for the assessment of factors regulating the acute release of CRH, TRH, and arginine vasopressin. We also employ an intravenously cannulated rat preparation with chronic maintenance and a system for maintenance of chronic central venous catheters in both the rat and non-human primate. Chronic intraventricular cannulae are also maintained in operantly conditioned non-human primates for studies of the behavioral effects of centrally-administered neuropeptides. Molecular methodologies include *in situ* hybridization, Northern blotting, transient and stable transfections, and standard cloning and sequencing procedures applied to the study of the type I glucocorticoid receptor and enkephalin genes.

Project Description: The following sections will outline our principal hypotheses regarding pathophysiological mechanisms in melancholic depression and Cushing's disease, our clinical and basic data regarding our hypothetical models, the significance of this work to the biomedical program of the institute and our future directions.

Background:

The principal stimulatory effectors of the generalized stress response consist of the corticotropin releasing hormone and the locus ceruleus-norepinephrine systems. Studies in

experimental animals show that CRH serves not only to activate the pituitary-adrenal axis, but also to coordinate a series of other physiological and behavioral responses adaptive during stressful situations. These include activation of the sympathetic nervous system and pathways subserving vigilance, cautious avoidance, and anxiety, and inhibition of vegetative pathways subserving growth, reproduction, sexual behavior, and feeding. Glucocorticoid secretion during stress serves primarily to counter-regulate the CRH and LC-NE systems to prevent pathological consequences of an unrestrained stress response. Glucocorticoids also participate in the inhibition of the hypothalamic-pituitary-gonadal axis at all levels, in the re-direction of energy, and in the restraint of the immune system.

Our data indicate that the clinical and biochemical manifestations of melancholic depression reflect an activation of the CRH and LC-NE systems that have escaped their usual counter-regulatory restraints. Moreover, our clinical and basic data suggest that the CRH and LC-NE systems participate in a mutual reverberatory positive feedback loop, and that each is directly or indirectly inhibited by many of the same stimuli, including glucocorticoids, opioid peptides, and gamma-amino-butyric acid. Dr. Brady will present her data showing that the chronic administration of the pharmacologic agent most effective in the treatment of melancholic depression (i.e. imipramine) serves to decrease the expression of CRH mRNA in the PVN and of tyrosine hydroxylase mRNA in the LC, in association with evidence of an increase in hippocampal glucocorticoid receptor-mediated restraint of the PVN CRH neuron. Dr. Kling will present data that CRH may play a role in the progressively exacerbating course of recurrent major affective disorder.

Based on the development of a series of methodologies that we applied in the diagnosis and evaluation of melancholic depression, Cushing's disease, and the various forms of adrenal insufficiency, we have established a means of estimating the functional integrity of each of the components of the hypothalamic-pituitary-adrenal axis. We have applied this methodology to the study of pathophysiological mechanisms in depressive illnesses associated with hypersomnia, hyperphagia, and fatigue that occur across the boundaries of psychiatric and medical disorders (e.g. seasonal affective disorder, Cushing's disease, hypothyroidism, and the Chronic Fatigue Syndrome). Our data suggest that each of these syndromes is associated with a pathological inactivation of the CRH neuron as a final common pathway that occurs via a variety of different pathophysiological mechanisms. In some instances, we have also advanced indirect evidence of an inhibition of the LC-NE as well. In addition, basic studies in progress have begun to identify systematic features of drugs preferentially effective in the treatment of atypical depression. As an example, Dr. Brady will report that in contrast to imipramine, the chronic administration of fluoxetine, phenelzine, and idosoxan each increase the expression of tyrosine hydroxylase in the LC.

Although our pre-clinical data suggest the possible involvement of hippocampal glucocorticoid receptors in the dysregulation of the stress response seen in melancholic depression, we cannot definitively account for the alterations in stress-responsive arousal producing neurotransmitter systems in the principal subtypes of major depression. However, we have conducted a series of studies exploring the effects of a variety of neurotransmitters, neuropeptides, inflammatory mediators, and steroid hormones on the secretion of CRH in an effort to identify possible factors promoting dysregulation of HPA function and developing improved means of therapeutic intervention.

Pathogenesis of Melancholia:

The idea that major depression reflects a pathological stress response is best reflected in the clinical presentation of melancholia, characterized by accentuation and prolongation of the hyperarousal characteristic of the generalized stress response. Experientially, this hyperarousal presents as an organized state of anxiety about the self, reflected in a pervasive loss of self-esteem and inappropriate guilt. Physiologically, there is a facilitation of arousal-producing neural pathways, evidenced by increased vigilance and focused attention, and by activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Melancholia is also associated with a concurrent inhibition of the neural pathways subserving vegetative functions such as reproduction, sexual behavior, and feeding.

We have advanced several lines of indirect evidence indicating that the hypercortisolism of melancholia reflects hypersecretion of CRH. First, we showed that the ACTH responses to synthetic CRH were attenuated in melancholia and correlated negatively with basal hypercortisolism, indicating intact glucocorticoid negative feedback upon the pituitary. This evidence suggestive of a central locus for hypercortisolism in depression appeared simultaneously with our findings that a continuous infusion of CRH to healthy volunteers could reproduce the 24-hour basal circadian pattern and the magnitude of hypercortisolism typically seen in melancholia, and with our finding that CSF CRH levels in patients with melancholia correlated positively with indices of basal hypercortisolism. We shall also present data regarding the circadian pattern of CRH secretion into the CSF and the pituitary-adrenal responses to the glucocorticoid antagonist RU 486 that further support this formulation. We will also present a series of clinical and basic studies supporting a potential role for CRH in the natural history of recurrent, progressively exacerbating affective illness.

The putative activation of the CRH system in major depression also seems associated with a concomitant activation of the LC-NE system. Accordingly, melancholic patients show normal or increased levels of norepinephrine in the cerebrospinal fluid (CSF), and increased levels of CSF and urinary 3-methoxy-4-hydroxy phenol glycol, a principal metabolite of norepinephrine. As a corollary, successful responses to antidepressant medication are consistently associated with decreases in CSF and plasma MHPG, while pre-clinical data indicate that the monoamine oxidase inhibitors and tricyclic antidepressant agents decrease the firing rate of the locus. Our data that CSF CRH correlates positively with CSF NE in volunteers and with a variety of indices of noradrenergic function in patients with depression supports the idea of a linkage between the activation of the CRH and LC-NE systems in controls and patients with melancholia. The idea of a concomitant activation of the sympathetic system in patients with melancholia is also supported by the findings of Dr. Mitchel Kling, who showed an increased rate of norepinephrine spillover into arterial plasma in patients with melancholia.

The idea that melancholia is associated with a concomitant activation of the CRH and LC-NE systems that have escaped their usual glucocorticoid-mediated counter-regulation is supported by the basic studies of Dr. Brady and her colleagues showing a chronic but not acute tricyclic antidepressant-induced suppression of the expression of the CRH gene in the PVN and of the TH gene in the LC, in association with a significant increase in type 1 GR mRNA in hippocampus.

One of the persistent problems in clinical neuroendocrinology has been determining the differential diagnosis between major depression and Cushing's disease. Indeed, because depression can present with hypercortisolism of the magnitude of that seen in Cushing's disease, while patients with Cushing's disease can present with major depression, the two entities can be impossible to distinguish from one another. On the basis of the overlapping clinical and

biochemical symptomatology, some have suggested that the two entities share pathophysiological features. In his 1973 Sir Henry Dale Lecture, Grant Liddle termed the problem posed by the differential diagnosis and pathophysiology of major depression and Cushing's disease as one of five enduring endocrinologic enigmas. We have conducted a series of studies to explore the differential pathophysiology and diagnosis of these two illnesses. The first of these involved exploring the functional integrity of the pituitary corticotroph cell by administering ovine CRH. In patients with depression, plasma ACTH's were attenuated in proportion to the degree of basal hypercortisolism, indicating that the pituitary corticotroph cell in this disorder was appropriately restrained by glucocorticoid negative feedback. On the other hand, patients with Cushing's disease showed exaggerated ACTH responses to ovine CRH despite basal hypercortisolism, indicating that the pituitary corticotroph cell was grossly unresponsive to the feedback effects of the glucocorticoids. These findings represented the first clinical data in which responses obtained in patients with depression and Cushing's disease went in the opposite direction, and the limited overlap in the responses rendered the ovine CRH test as an extremely useful one in ascertaining the differential diagnosis of these entities. To date, our data show less than 18% overlap between the two groups.

Studies of hypothalamic-pituitary-adrenal axis function in patients with melancholia:

Basal diurnal pattern of pituitary-adrenal function in depression: 36-hour sampling of plasma cortisol in major depression:

Our studies examining plasma cortisol secretion by serial blood sampling over a 36-hour period, encompassing two consecutive nights, show that mean plasma cortisol levels are significantly elevated in depressed patients compared with healthy volunteer subjects. In addition, not only are there no significant differences between the plasma cortisol levels on the first and second nights in both depressed patients and healthy volunteers, but the patterns of secretion are strongly correlated between the two nights in both groups, suggesting that the elevated plasma cortisol levels in the depressed patients are robust and not due merely to nonspecific stress, novelty of the procedure, or other instrumental variables. The elevation in plasma cortisol levels occurs throughout the 24-hour cycle, but is most apparent during the evening hours, when cortisol secretion is often not detectable in controls but continues to occur in depressed patients. These findings are more consistent with an early activation of the pituitary-adrenal axis in depression than with a phase-advance of the entire rhythm, as had been previously proposed, and are compatible with circadian physiology data indicating that the mechanisms which govern the onset and offset of such rhythms are distinguishable from 36-hour pattern of plasma ACTH secretion in major depression.

In contrast to elevated plasma cortisol levels in melancholic depression, we found that the mean 36-hour plasma ACTH levels were only slightly and not significantly elevated in the depressed patients (although slightly more so in the late evening). Normal overall plasma ACTH levels in depression are compatible with our previous data showing that the adrenal cortex in melancholic depression has become hyper-responsive to ACTH over the course of a sustained, centrally-driven hypercortisolism; hence, because it is the central nervous system that determines the set point for the cortisol production rate, a smaller pulse of ACTH is required to produce the centrally-mediated hypercortisolism from the adrenals that have grown hyper-responsive to ACTH. However, in the context of adrenal hyper-responsiveness, such a "normal"-amplitude pulse of ACTH is actually inappropriate, and indicates an alteration in the central set-point for pituitary-adrenal regulation.

Requirements and rationale for defining pulsatile ACTH and cortisol secretion in depression.

While these initial studies provided useful data regarding the temporal pattern of ACTH and cortisol secretion in depression, they did not allow us to unequivocally assess the frequency and amplitude characteristics of discrete secretory pulses of ACTH and cortisol, as we had initially hoped, because of a lack of information, either from our own data or from the literature, regarding the decay rates of these hormones. Such information is necessary in order to use deconvolution methods to resolve overlapping peaks. Studies are now ongoing to determine these decay rates empirically, both in healthy subjects and in patients with melancholic and nonmelancholic depressions, and subsequently to define the architecture of pulsatile ACTH, cortisol, and arginine vasopressin (AVP) secretion in these subjects (see Future Directions). Such studies will help answer the important questions of whether the disposition of ACTH and cortisol differs in patients and controls, which is essential in order to accurately interpret steady-state plasma levels. These studies are also essential for making inferences regarding ACTH and cortisol production rates, the effects of immediate and delayed glucocorticoid negative feedback on ACTH pulse characteristics, and whether different kinds of ACTH secretory episodes may occur, perhaps at different times of day, with distinct amplitude and duration characteristics reflecting the actions of different secretagogues (e.g., CRH alone vs. CRH plus AVP).

30-hour pattern of neurohormones and neurotransmitters in the CSF of patients with affective illness and controls:

Data from several studies in our group and others suggest that the hypercortisolism of melancholic depression derives from a locus at or above the hypothalamus resulting in the hypersecretion of CRH. In contrast, the hypercortisolism of Cushing's disease is associated with a pituitary defect resulting in the hypersecretion of ACTH. Given these data, we have sought to examine the relevance of this CRH hypersecretion to the symptom complex and longitudinal course of major affective illness. To this end, we have shown that CSF CRH levels are inappropriately elevated in patients with major depression when compared with patients with Cushing's disease showing comparable degrees of hypercortisolism. These findings are consistent with other data from our group showing single time point CSF CRH levels to be positively correlated with postdexamethasone cortisol levels in patients with major depression, further suggesting that their hypercortisolism is associated with hypersecretion of CRH.

In collaboration with Dr. Edward Oldfield in the Surgical Neurology Branch, NINDS, we have attempted to further explore the regulation and dysregulation of CRH and related neurohormones in the central nervous system by examining their CSF levels around the clock by continuous sampling of CSF through an indwelling lumbar catheter. We felt that such an invasive procedure was both safe and justifiable for the following scientific reasons: (1) nearly all, if not all, data concerning CSF neurohormone levels in depression were based on single time point lumbar punctures at 9 a.m. that could easily miss functionally important differences; (2) the nonuniform pattern of cortisol secretion throughout the day most likely reflected a diurnal rhythm of CRH secretion whose characterization was essential for the adequate clinical study of depression; (3) this procedure afforded a unique opportunity to examine the dynamics of neurohormonal secretion into the CSF that was not only of intrinsic physiologic interest but also critical to the question of whether neurohormonal secretion into the CSF was of functional or pathophysiological relevance; (4) this procedure afforded the opportunity to explore the relationship between the secretion of neurohormones into the CSF and the physiology of circadian pituitary secretion both in healthy controls and in depressed patients; and (5) this procedure allowed definition of dynamic interrelationships in the CSF secretion of a number of neurohormones that are functionally related and that have been implicated in the

pathophysiology of major depression [e.g. not only CRH, but also AVP, oxytocin (OT), somatostatin (SRIF), catecholamines (CA), and serotonin (5-HT)].

To date, we have studied 6 healthy volunteers and 6 depressed patients. Thus far, we have found that the CSF levels of CRH show a statistically significant diurnal rhythm which is nearly opposite in phase to that of plasma cortisol in both healthy volunteers and patients with melancholic and psychotic depression; hence, the CSF CRH pattern was characterized by significantly greater secretory activity during the evening and early morning hours, when plasma cortisol secretion is at its nadir. This finding, while unexpected, has now been found to be consistent with data from studies in nonhuman primates showing a diurnal rhythm of CSF CRH levels which is of opposite phase to that of cortisol secretion. One possible explanation for this finding is that the CRH levels in the CSF are suppressed by the peripheral cortisol rhythm with a time-lag corresponding to the transcriptional and/or translational effects of glucocorticoids which may be needed to effect these changes. Alternatively, there may be multiple sources of CRH in the CSF, some of which reflect secretion not directly correlated with hypothalamic CRH. Further studies are needed to clarify the relationship between CSF CRH and the functional activity of brain CRH systems. Nevertheless, we have observed a significant fall in CSF CRH levels measured throughout the 24-hour cycle in 2 of 2 patients with severe melancholic depression associated with significant hypercortisolism following successful treatment with electroconvulsive treatment (ECT), suggesting a pathologic elevation in centrally-directed CRH secretion prior to treatment.

Effects of the glucocorticoid antagonist RU 486 on pituitary-adrenal function in depression:

Further data supporting the involvement of CRH in the hypercortisolism of depression comes from studies using the glucocorticoid antagonist RU 486 as a probe of glucocorticoid negative feedback in patients with mild to moderate major depression. RU 486 has previously been shown to produce a dose-related disinhibition of pituitary-adrenal function in healthy subjects which occurs principally during the early morning hours, presumably reflecting active secretion of CRH during these times. In patients with major depression given RU 486, the activation of plasma ACTH and cortisol secretion occurs at least one hour earlier than in control subjects, compatible with our previous serial sampling data showing early activation of spontaneous ACTH and cortisol secretion in depressed patients. These data suggest that RU 486 can unmask this tendency toward early activation of ACTH secretion, even when basal pituitary-adrenal function is relatively normal. These data also suggest that primary glucocorticoid resistance is unlikely to play a physiologically significant role in the hypercortisolism and dexamethasone resistance of depression, as had been proposed by some previous studies. Hence, if significant glucocorticoid resistance were present, the ACTH response to RU 486 should be blunted. Moreover, the diurnal rhythm in CRH secretion should be fundamentally preserved if glucocorticoid resistance were present, so that ACTH secretion would not be activated in depressed patients at times when it was not provoked in healthy subjects.

Relevance of CRH to basal and stress-mediated human pituitary adrenal function:

In support of the possible relevance of CRH to the hypercortisolism of major depression, we first demonstrated that CRH was of relevance to human pituitary-adrenal function. Our data show that the pulsatile administration of human CRH designed to mimic endogenous ACTH pulsatile secretion restored normal basal circadian pituitary-adrenal function in patients with central adrenal insufficiency. We also first demonstrated that the pituitary corticotroph cell, like the pituitary gonadotroph, requires the priming effect of endogenous or synthetic CRH in order to respond appropriately to subsequent CRH stimulation. On the other hand, lest we conclude that CRH is the only factor involved in the hypercortisolism of depression, our data and those of

others indicate that factors other than CRH are involved in pulsatile ACTH release; as an example, we demonstrated that healthy volunteers given a continuous infusion of ovine CRH continue to show pulsatile ACTH release, suggesting that while CRH sets the amplitude of ACTH pulses, other factors (e.g. the pulsatile release of vasopressin) influence the pulse frequency of pituitary-adrenal function. Furthermore, in studies of stress-mediated pituitary-adrenal activation, we showed that the HPA axis is activated in an exercise intensity-dependent fashion, correlating well with both the percent of maximal O₂ consumption (VO₂max) and plasma lactate concentrations. While our *in vitro* hypothalamic organ culture studies show that lactate causes the dose-dependent release of CRH, the lowest maximal stimulatory dose is lower than that achieved in plasma during exercise, suggesting that lactate and other factors promote ACTH release not only through CRH, but other factors as well.

Exploration of norepinephrine kinetics in patients with affective illness:

Previous data had suggested a positive relationship between CSF CRH and indices of noradrenergic function in depressed patients. In order to further explore noradrenergic function in depression, we have recently conducted studies examining the spillover of NE into arterial plasma during a continuous infusion of physiologically-inert doses of 3H-NE given to measure the rate of clearance of NE from plasma. Previous studies of plasma norepinephrine in depression have almost exclusively relied on measurements taken from a forearm vein. However, this measure has been shown to be heavily influenced by local release and uptake of norepinephrine in the extremity from which samples are taken. Moreover, plasma levels of NE are determined, not only by the rate at which NE "spills over" from its release sites into plasma, but also by its clearance from plasma. The latter, which is a function of both neuronal and nonneuronal uptake, is strongly correlated with cardiac output, which may show considerable variation both within and between subjects. Hence, the measurement of arterial plasma NE provides a better reflection of whole-body NE release, while simultaneous measurement of the clearance of NE allows estimation of the actual rate of spillover of NE into the peripheral circulation. This technique has been shown not only to be more sensitive to changes in total-body NE output than standard methods utilizing forearm venous NE, but also to show abnormalities in individuals with a family history of essential hypertension who were themselves normotensive. To date we have studied 13 patients with major depression and matched control subjects. Our preliminary data indicate that NE spillover is significantly increased in a subgroup of 5 patients with melancholic depression compared with controls.

In addition to determining the basal NE spillover rate, we also attempted to determine the spillover rate in response to an environmental challenge. We selected responses measured during the playing of a video game that had previously differentiated normotensive controls with and without family histories of hypertension. In this regard, one of our principal theoretical challenges is to determine whether patients with a susceptibility to the hyperarousal of melancholic depression show an intrinsic hyper-responsiveness to environmental stress. Our models predict that this would be the case in the non-depressed patient with a history of melancholia. On the other hand, the established hyperarousal of the melancholic state might both clamp the stress-responsive arousal producing systems at maximal levels of stimulation so they would be unresponsive to external stimuli, analogous to the relative unreactivity of mood in melancholic depressives to external stimuli. Moreover, the profound hypercortisolism of melancholia might also restrain stress-responsive-arousal producing neurotransmitter systems from further upward excursions in the context of environmental stressors.

A third component of our norepinephrine spillover protocol has been the assessment of norepinephrine spillover rates during an intravenous infusion of yohimbine. This alpha-2

adrenergic antagonist has been shown to increase peripheral NE spillover in volunteers by a predominantly central effect of increasing sympathetic outflow. This central nervous system-derived bioassay for alpha-2 responsivity will allow us to more directly test the hypothesis that some patients with depression show altered sensitivity of alpha-2 receptors, compared with previous studies of platelet alpha-2 receptors which may or may not reflect the status of these receptors in the central nervous system. Our preliminary data show differences in the responsiveness of mood to the administered yohimbine between patients and controls, with reported anxiety showing the expected increase in controls but actually decreasing in the depressed patients in association with transient relief of depression in some patients and even mild hypomania in a few. Responses of plasma NE spillover to yohimbine are robust in both patients and controls but data are too preliminary at this time to allow comparison of magnitude of the response in the melancholic patients.

Also, in collaboration with Dr. Margaret Altemus, I have adapted this procedure to explore arterial NE spillover in patients with eating disorders and to determine, for the first time, the effects of food ingestion on peripheral NE kinetics. The latter is of interest in the light of data that the sympathetic nervous system is principally involved in promoting the increase in metabolic rate that occurs as a consequence of acute food intake and chronic weight gain. This technique allows us to test the hypothesis that patients with eating disorders show a deficient sympathetic response to food ingestion which may produce an enhanced proclivity towards weight gain, thus accounting for their use of food restriction and/or purging for weight control.

Putative interactions between the CRH and LC-NE systems

The weight of our available data and that of others suggest that the hypothalamic CRH neuron and the PVN participate in a reverberatory positive feedback loop that may be of relevance to the pathogenesis of melancholic depression. In our JCI paper of March, 1989, we settled a long-standing debate regarding the direct effects of NE on CRH release by conducting an extensive series of experiments to show that NE caused the acute release of CRH from PVN CRH neurons. Conversely, we also showed that the ICV administration of CRH causes a rapid, preferential increase in LC glucose utilization. Dr. Mark Smith in our group has report on his data that acute, but not chronic, stress increases the expression of CRH mRNA in the PVN and TH mRNA in the LC, while Dr. Linda Brady has reported on her data regarding the effect of chronic imipramine treatment to reduce the expression of the CRH gene in the PVN and of the TH gene in the LC. Others have shown that CRH increases the LC firing rate, that CRH antisera attenuates the effects of many stimuli that ordinarily increase LC firing, and that noradrenergic antagonists attenuate the behavioral effects of centrally administered CRH. On the other hand, Dr. Brady has shown that activating antidepressants such as MAO inhibitors show a dissociation of effects on tyrosine hydroxylase mRNA expression in the locus ceruleus and CRH mRNA expression in the paraventricular nucleus. Moreover, Dr. Brady and her colleagues have shown that destruction of the locus ceruleus is not associated with a long-term decrease in the expression of CRH mRNA in the paraventricular nucleus. Hence, our data is not uniformly consistent regarding concordance in the functional activity of the PVN CRH and LC-NE systems, and Dr. Brady plans further studies, including efforts to examine the effects of a variety of antidepressants in rats with bilateral electrolytic lesions of the locus ceruleus.

Studies on the potential role of CRH in the natural history of recurrent, exacerbating affective disorder.

In collaboration with Robert Post, we have previously shown that the icv administration of CRH to the rat produces limbic seizures that cross-sensitize with electrically-kindled seizures. On the basis of these data and a series of additional studies presented by Dr. Kling, we suggested that CRH might play a role in the exacerbating, recurrent course of major affective disorder. Among these findings include Dr. Kling's data that in contrast to controls who show a negative correlation between CSF CRH and age, patients with melancholia show a positive correlation between these parameters. These findings are compatible with our previous data and those of others showing that in contrast to controls, patients with major depression show an increase in mean 24-hour urinary free cortisol excretion with advancing age.

To further explore the potential role of CRH in kindling and sensitization phenomena in clinical studies, in collaboration with Dr. Robert Post, we administered procaine to volunteers and patients with affective disorder to monitor pituitary-adrenal and behavioral responses. We noted that procaine produced dose-dependent increases in ACTH and cortisol secretion in both patients and volunteers in association with mood and/or psychosensory changes.

To explore the potential mechanism of procaine's effects upon the pituitary-adrenal axis, we assessed the effects of this agent as well as other local anesthetics such as lidocaine and cocaine on in vitro release of CRH from hypothalamic and of ACTH from cultured pituitary cells. We noted that procaine produced a dose-dependent increase in CRH release but had no effect on the pituitary corticotroph. Interestingly, the effect of procaine was blocked by carbamazepine but not by a number of other agents, including alpha 1 and alpha 2 blockers, serotonin 1B blockade, and nicotinic and muscarinic blockade.

We also noted that carbamazepine was capable of inhibiting the responsiveness of the CRH neuron to a variety of stimuli, though it had no intrinsic effect on the CRH neuron when given alone. Moreover, we showed that carbamazepine had a weak direct stimulatory effect upon the pituitary corticotroph cell. These findings probably account for our in vivo data that carbamazepine administration to non-stressed rats causes a moderate increase in pituitary-adrenal function, but when given to stressed rats, produces a decrease in pituitary-adrenal function. Similarly, carbamazepine administration to normals causes a slight increase in plasma ACTH and cortisol secretion. When given to depressed patients who are hypercortisolemic, carbamazepine decreases plasma ACTH and increases the pituitary-adrenal response to exogenous CRH; conversely, when given to eucortisolemic depressed patients, basal ACTH and cortisol values rise in association with a fall in plasma ACTH and cortisol secretion during the administration of CRH. These data suggest that one possible mechanism of carbamazepine's capacity to exert prophylactic efficacy in affective disorder is to buffer stress responsive systems and promote the establishment of an equilibrium in their functional activity.

To further explore the potential role of CRH on neuronal kindling, we pretreated rats with icv CRH antisera. Our preliminary data show that this intervention was able to attenuate the development of electrically induced kindling. Taken together, these data suggest that repeated activation of the CRH system over the course of recurrent depression could sensitize underlying limbic substrates to influence the natural history of the illness and supports the idea that a principal neuropeptide involved in promoting behavioral and physiological arousal is involved in a process which is known to show cross-sensitization with stressful stimuli. To follow up on these findings, Drs. Mark Smith and Harvey Whitfield have embarked upon a series of studies to explore the effects of electrical kindling on the expression of genes of interest utilizing in situ hybridization. Our data to date show that electrical kindling causes a significant increase in CRH

mRNA expression in the amygdala lasting long after the last stimulus is applied. This long-lasting effect on the expression of the CRH gene in the limbic system is of potential interest in the light of our postulates regarding the role of CRH in affective disorder and in the therapeutic actions of carbamazepine.

We have also shown that electrical kindling increases the expression of CRH mRNA in hippocampal interneurons where it is not ordinarily expressed. These are GABAergic interneurons in which CRH is now co-expressed. In the light of data that CRH inhibits the release of GABA from neurons in which the two peptides are co-expressed, we suggest that CRH may play an excitatory role under these conditions by counter-regulating GABAergic neurotransmission in the hippocampus, in keeping with a possible generalized excitatory role that CRH seems to play in disparate CNS structures.

Studies of the Effects of Aging on the Hypothalamic-Pituitary-Adrenal Axis of Relevance to Melancholia

Our data show that aging in the rat is associated with a progressive hypothalamic CRH deficiency producing a mild tertiary adrenal insufficiency. This finding further highlights the possible significance of the gradual increase in CRH secretion into the CSF with age in patients with major depression. Our *in vitro* data supporting this central adrenal insufficiency include an age-dependent progressive decrease in CRH mRNA expression and content in the PVN as well as in CRH release from hypothalami taken from aged rats; a decrease in pituitary POMC mRNA expression and ACTH content in the pituitary of aging rats in association with an increase in pituitary CRH receptors and in the response of dispersed pituitocytes to synthetic CRH (presumably as a consequence of prior decreased stimulation by endogenous CRH, and a decrease in adrenal weights and decreased adrenal responsiveness to ACTH 1-24. Our corroborating *in vivo* data include diminished responsiveness to parenteral administration of the central CRH stimulus IL-1, decreased plasma ACTH responses to arginine vasopressin infusion, and decreased adrenocortical responses to exogenous ACTH.

Regulation of CRH Secretion

In an effort to both further elucidate the regulation of the CRH neuron and to advance data regarding the possible factors that could promote CRH hypersecretion in melancholia, we developed a rat hypothalamic organ culture system with which we studied the *in vitro* regulation of dose-dependent hypothalamic CRH secretion directly. The viability of this model has been extensively validated by electron-microscopy and many other techniques, and by corroborative *in vivo* studies (see fig. 1 below for summary of results)

Excitatory Stimuli:

Neurotransmitters and Neuropeptides: We have shown that norepinephrine (via the alpha 1 and 2 receptors), neuropeptide Y, (which is co-secreted with norepinephrine), serotonin (via 1A and 5HT 2 receptors), and acetylcholine (via both muscarinic and nicotinic receptors) stimulate CRH secretion. Vasopressin not only acts synergistically with CRH at the pituitary corticotroph cell and in the brain, and is co-secreted with CRH under some circumstances, but also stimulates the release of CRH via V2 receptors.

Cytokines and Eicosinoids: We have shown that epidermal growth factor, platelet activating factor, tumor necrosis factor, and several eicosinoids are potent stimuli to the CRH neuron. We have also replicated the work of others with our experimental model showing

stimulatory effects of IL-1. The responses to IL-1 and TNF- α could be inhibited by prostaglandin synthesis blockade. None of these substances could stimulate pituitary ACTH secretion in vitro during 4h incubations at concentrations lower than 10-7M. As Dr. Sternberg will note, these substances could reflect links between the immune-inflammatory response and the CNS, though they may also play an auto/paracrine role in the regulation of the CRH neuron.

Inhibitory Stimuli:

Neurotransmitters: GABA inhibits the release of CRH via both GABA A and GABA B receptors. Benzodiazepines also restrain the CRH neuron. Alprazolam, which is also a PAF antagonist, is 100 times more potent than diazepam.

Feedback regulators: Glucocorticoids inhibit CRH release in a classic negative feedback loop. Arcuate nucleus POMC fragments (including ACTH, b-endorphin, alpha MSH and CLIP), inhibit CRH release via short-loop negative feedback. Using a human-rat CRH antisera that did not cross-react with ovine CRH, we also showed that CRH restrains its own release via an ultra-short negative feedback loop.

In vivo corroboration: We have obtained corroborative in vivo data from many of our in vitro studies in experiments with chronically cannulated rats. These experiments were conducted in association with the in vivo administration of CRH antisera and in conjunction with in vitro pituitary cell culture and hypothalamic organ culture studies (for vasopressin release) to determine the mechanisms by which various pharmacologic agents act on the HPA axis. As an example, our data indicate that 5HT 1A and muscarinic agonists act specifically upon the CRH neuron to promote pituitary-adrenal activation and can thus be used as probes of hypothalamic CRH responsiveness in melancholia and other states. By utilizing either dexamethasone suppression and/or hypophysectomy, we have also shown that neuropeptide Y secreted by the adrenal medulla promotes glucocorticoid secretion directly and sensitizes the adrenocortical response to ACTH during stress.

Studies of the type I glucocorticoid receptor

We have long been interested in the functional significance of the type 1 GR on the basis of our interests in the central regulation of the pituitary-adrenal axis and the role of glucocorticoids as principal counter-regulatory elements of the generalized stress response. Our study of the type 1 GR was facilitated by Dr. Harvey Whitfield, who developed a technique for the accurate estimation of type I mRNA by in situ hybridization in a large number of samples. Utilizing this technique, Dr. Brady advanced data that the type 1 GR in the hippocampus participated in the capacity of chronic tricyclic drug administration to counter-regulate the activation of the generalized stress response seen in melancholic depression. We have also shown the presence of type I mRNA in primate hippocampus in a distribution similar to that seen in the rat, suggesting that this receptor may play an important role in the human HPA axis, while Dr. Sternberg and Dr. Whitfield have conducted studies of the ontogeny of this receptor suggesting that its brief expression in the hypothalamus early in life may be responsible for the stress-unresponsive period in the rat.

Significance to the biomedical research program of the institute:

We have attempted to delineate physiological, biochemical, and molecular mechanisms of physical and emotional stress and their relevance to major psychiatric illness. Accordingly, our basic clinical studies focus is on stress-responsive neuromodulators. Our work with one of the major effectors of the stress response, namely corticotropin releasing hormone, illustrates, in part, our approach to these goals. In a series of over 10 papers in The New England Journal of Medicine, 8 in the Journal of Clinical Investigation, and over 25 in the Journal of Clinical

Endocrinology and Metabolism we accomplished the following : (1) advanced the first data that CRH was of physiological relevance to human pituitary-adrenal function; (2) established that stress-induced pituitary-adrenal activation in humans required factors other than CRH; (3) showed that the pituitary corticotroph required the priming effects of its hypothalamic releasing factor to function properly; (4) compared and contrasted the pharmacokinetic and biological properties of ovine and human CRH; (5) showed that ovine CRH was best suited for diagnostic testing while human CRH could be used clinically to explore pulsatile ACTH secretion; (6) performed dose-response studies for human and ovine CRH and administered them at different times of day to work out the conditions for a clinically applicable CRH stimulation test; (7) applied the CRH stimulation test to work out the differential diagnosis between major depression and Cushing's disease, Cushing's disease from ectopic ACTH secretion, and secondary from tertiary adrenal insufficiency; (8) elucidated pathophysiological mechanisms in the hypercortisolism of major depression and Cushing's disease and proved that these two illnesses represented distinct pathophysiological processes; (9) showed that the hypercortisolism of major depression and anorexia nervosa had a similar pathophysiological basis attributable to CRH which could, in turn, contribute to the common clinical and biochemical manifestations of these illnesses; (10) developed hypothetical models for the potential role of CRH in other psychiatric illnesses characterized by hypercortisolism, including panic disorder; (11) elucidated the role of serotonin, norepinephrine, acetylcholine, GABA, benzodiazepines, excitatory amino acids, and feedback regulators such as beta-endorphin, ACTH, and CRH itself on the regulation of hypothalamic CRH release. All efforts to replicate this work have so far been successful, and the fruits of this work have been translated into everyday clinical practice in centers all over the world. In addition, our hypotheses regarding the etiology of major psychiatric illnesses such as melancholia are among the most intensively pursued areas of investigation in psychiatric research.

In a two-part series recently published in *The New England Journal of Medicine*, we proposed that the clinical and biochemical manifestations of major depression reflected the concomitant activation of the two major stress-responsive neurotransmitter systems in brain that had escaped their usual glucocorticoid-mediated counter-regulation. This hypothetical model, best supported by our own original data first published in *The New England Journal of Medicine*, has become the basis for perhaps the most intensively pursued hypothesis regarding pathophysiologic and etiologic mechanisms in major depression. An adjunct to these studies has been a series of basic studies implicating an important role for CRH in sensitizing key substrates in brain, and hence, in influencing the natural history of recurrent affective illness. Moreover, in a recent paper published in *The Journal of Clinical Investigation*, in which one segment of the data served as the cover photograph, we showed that the molecular effects of chronic but not acute antidepressant medication involve a consistent decrease in the expression of the CRH gene in the hypothalamus and of the TH gene in the locus ceruleus, in association with an increase in the expression of the type 1 glucocorticoid receptor in the hippocampus, thought to be a principal restraining element of the CRH system. These data nicely complement our original hypothesis that melancholia represents an activation of the generalized stress response that has escaped its counter-regulatory influences by showing that chronic, but not acute tricyclic antidepressant treatment restrains the principal components of the generalized stress response.

Dr. Grant Liddle in his 1973 Sir Henry Dale Lecture highlighted the question of whether the hypercortisolism in major depression and Cushing's disease represented similar or distinct pathophysiological questions, and termed this one of four enduring endocrinological enigmas. This question remained unanswered until we approached it in our clinical applications of corticotropin releasing hormone, when we showed that the hypercortisolism of major depression represented a defect at or above the hypothalamus resulting in the hypersecretion of CRH, while the hypercortisolism of Cushing's disease represented inadequate glucocorticoid restraint upon

the pituitary. Our data showing suppression of the hypothalamic CRH neuron in patients with Cushing's disease clarified the mechanism of the post-operative adrenal insufficiency in this disorder, provided the rationale for therapeutic interventions, and established the hypothesis that the atypical depression seen in Cushing's disease could reflect a pathological inactivation of the CRH neuron. Parenthetically, our clinical applications of CRH also contributed to the development of improved means for the differential diagnosis of primary, secondary, and tertiary adrenal insufficiency, and of Cushing's disease from ectopic ACTH production.

Our basic science laboratory first suggested what has now been further validated by a number of laboratories; namely, that the locus ceruleus-norepinephrine systems participate in a mutual reverberatory positive feedback loop, in which each reinforces the other's functional activity. To explore the regulation of the CRH component of this loop, we have performed a series of studies exploring the neurotransmitter, neuropeptide, feedback, and cytokine regulation of the CRH neuron. These studies represent the most comprehensive series of studies to date elucidating the neurotransmitter, neuropeptide, cytokine, and feedback modulation of the central component of the hypothalamic-pituitary-adrenal axis, and raise theoretical questions regarding potential mechanism of action of psychotropic agents, the treatment of steroid-induced adrenal insufficiency, and the treatment of stress-induced hypothalamic amenorrhea and infertility occurring as a consequence of a shortened luteal phase.

Future Directions

Studies of the Pulsatile Secretion Of Hormones into Plasma and CSF

We propose studies which will define the pulsatile architecture of ACTH and cortisol secretion in patients with depressive syndromes and healthy volunteers. Such studies are not only of intrinsic physiological interest, but will also help us to further clarify the central mechanism underlying alterations in pituitary-adrenal function in patients with psychiatric disorders. In this regard, preliminary data from experimental animals suggest that the diurnal pattern of ACTH secretion reflects a pattern of regular pulses of CRH secretion which may or may not be accompanied by pulsatile AVP secretion. We also know that AVP acting jointly with CRH produces pulses of greater amplitude and duration than those produced by CRH alone. Thus, precise measurement of ACTH pulse characteristics throughout the 24-hour cycle may allow inferences regarding the pattern of hypothalamic CRH and AVP secretion in humans. To accomplish this task, it is necessary to determine the instantaneous secretory rates (ISRs) for ACTH, AVP, and cortisol from q 10-15' serial blood sampling. These ISRs are heavily dependent on knowledge of the decay rates of ACTH and cortisol in plasma after secretion, as the instantaneous level of a substance in plasma is determined both by its production and clearance rates. To determine the clearance rates we will administer cortisol, synthetic ACTH1-39, and AVP to patients and volunteers after overnight suppression of endogenous secretion with 4-8 mg of oral dexamethasone. The decay rates will be fitted by standard nonlinear regression techniques to either a uni- or biexponential curve, the choice between which will be made by goodness-of-fit tests. We will then, on a separate occasion, conduct 24-hour serial blood sampling for ACTH, AVP, and cortisol. The data will then be subjected to deconvolution analysis, using the individual's empirically-determined decay parameters, to estimate the ISRs for these hormones as a function of time which can then be analyzed for the presence of discrete peaks using our modification of the program DETECT, initially developed by Dr. David Rodbard and colleagues. The actual amount of hormone production over the 24-hour period sampled can then be estimated for each individual, based on their calculated volume of distribution and integration of the ISR-time curve. In the case of cortisol, the accuracy of these measurements

will be externally validated against a method recently published by Drs. Nora Estoban and Alfred Yergey of NICHD which estimates cortisol production rate during a continuous infusion of tracer amounts of deuterium-labeled cortisol and mass-spectrographic separation of native and deuterated cortisol in plasma samples. The availability of these data will also enable us to develop and test models of the negative feedback effects of prevailing cortisol secretion on the characteristics of endogenously-secreted ACTH pulses.

As a corollary to this study, we will compare the respective correlations of our two assays for ACTH. The first is our standard radioimmunoassay, which uses a single antibody directed at the mid-portion of the ACTH molecule, and which may detect a variety of ACTH fragments (some of which, in proportions unknown at this time, may be biologically inactive). The second is a more specific immunoradiometric assay (IRMA) for ACTH1-39 (which uses two antibodies directed at opposite ends of the molecule, and thus, while eliminating detection of biologically inactive fragments, may fail to detect fragments which are cleaved near the C-terminal of the molecule and thus retain biological activity). In addition, the results obtained with each assay will be correlated with bioassayable ACTH using an adrenal cell culture system which has been developed in our laboratory.

2) We propose to continue studies utilizing serial CSF and concurrent blood sampling. Our specific future aims include:

a) determination of whether the diurnal pattern of CRH we have measured represents true circadian architecture by examining whether the rhythm of CSF CRH persists in the absence of environmental and social cues which ordinarily either entrain circadian rhythms to 24-hour cycles (zeitgebers) or give the false impression of a circadian rhythm (masking effects). These studies will be done in collaboration with Dr. Wehr and colleagues using techniques they have developed and used for observing free running circadian rhythms and removing masking effects, such as maintaining constant lighting conditions, hourly or random schedules of feeding, and removal of clocks, external light sources, and other means of assessing real time. These studies can be readily done in healthy subjects at the NIH under a unique program whereby student normal volunteers are admitted to the inpatient unit for many weeks at a time. Under these conditions, it will also be possible to determine whether or not the CSF CRH and peripheral cortisol rhythms are internally synchronized by observing whether or not they dissociate from each other under constant conditions, as do other rhythms such as rest-activity and temperature.

b) Examination of whether diurnal rhythms of CRH can be detected in patients with Cushing's disease, who show near-total suppression of hypothalamic CRH. Although this could be tested in nonhuman primates given chronic glucocorticoids, the problems of maintaining catheters for CSF sampling long-term in such animals may actually make it easier to conduct such studies in patients. We note in this regard that continuous CSF sampling has been conducted in patients with metastatic cancers, who are severely immunocompromised, without any infectious or other complications except for a typical post-LP headache in a few subjects.

c) We propose to examine the CSF pattern of NE and other CA in healthy subjects given ganglion blockers (which have been safely used in studies of peripheral NE kinetics) to assess the contribution of peripheral sympathetic innervation to CSF NE levels.

d) We will explore the feasibility of using the technique of continuous CSF sampling to examine the kinetics of:

(1) NE using continuous infusion of deuterium-labeled tyrosine, which will be incorporated into deuterated NE only within the CNS (pending the outcome of studies in 2(c); we

can thus measure rate of appearance of brain-derived catecholamines; we can also assess the relative contribution of peripheral and central CA to CSF catechol metabolites.

(2) 5-HT after bolus ingestion or infusion of deuterium-labeled tryptophan - follow appearance of 2H-serotonin, melatonin (simultaneously secreted into plasma and CSF), 5HIAA, kynurenine, quinolinic acid etc. in plasma and CSF - assess baseline and after treatment with 5-HT agonists and uptake blockers.

e) We shall also explore dynamic changes in the CSF secretion of CRH and other neurohormones of theoretical interest, including AVP, after provocative challenges including:

(1) cholinergic agents since chronic atropine has been reported to up-regulate CRH receptors in cortex, suggesting that cholinergic agonism stimulates CRH, and physostigmine has already been reported to induce acute changes in CSF monoamines.

(2) hypertonic saline and glucoprivic stimuli such as insulin-induced hypoglycemia or 2-deoxyglucose, which have been shown to stimulate peripheral AVP secretion and some of which may also stimulate CRH secretion.

NE Kinetics in Patients with Melancholia:

Further studies of peripheral NE kinetics in affective illness, including examination of the acute and chronic effects of antidepressants, especially serotonergic drugs which we have also shown also to affect CSF NE metabolites and have antidepressant, anxiolytic, and antipanic properties. These data would be compared with data from parallel collaborative studies in patients with other disorders in which noradrenergic dysfunction has been shown or suspected, including chronic fatigue and panic-anxiety disorders (panic disorder, social phobia, PTSD, OCD). Study of such patient groups will not only help us to more precisely define peripheral noradrenergic function in these conditions, but also to further examine the relationship or lack thereof between noradrenergic and pituitary-adrenal status in these patients. Hence, patients with panic disorder show some evidence for noradrenergic and pituitary-adrenal dysfunction, although this has not been consistently demonstrated; patients with PTSD may have increased NE responses to stressful stimuli, and also show blunted ACTH responses to CRH with relatively normal evening cortisol levels and low urinary free cortisol excretion. Similarly, patients with OCD show increased CSF CRH with apparently normal peripheral cortisol measures; noradrenergic status has been incompletely examined.

Continuous Drainage of Lumbar CSF:

We propose to continue our studies of cerebrospinal fluid neurohormones obtained from lumbar puncture in patients with depressive syndromes to examine for potential alterations in the CSF levels of newly-discovered neurohormones in the pathophysiology of these syndromes. For example, we have recently shown reductions in cerebrospinal fluid immunoreactivity for endothelins (ETs), a family of potent vasoconstrictor peptides, in patients with major depression. ETs and their receptors have recently been shown to be widely distributed in brain, including the hypothalamus and neurohypophysis, and to have central effects on behavior and physiology suggesting a close physiologic relationship between these peptides and both NE and other stress responsive and counterregulatory neurohormones such as CRH, vasopressin, and atrial natriuretic factor in brain.

Our continued study of CSF neurohormones in Cushing's disease will aid in the process of identifying substances which may be involved in the pathophysiology of depressions by

providing information on the effects of glucocorticoid excess per se on the CSF neurohormonal milieu. Hence, in contrast to SRIF, the CSF levels of which are even more reduced in Cushing's disease than in major depression, the CSF levels of endothelin are reduced to a lesser extent in Cushing's disease than in major depression, suggesting that hypercortisolism alone cannot account for the magnitude of ET reduction in depression.

Additional studies will be conducted to examine for the presence in CSF of individuals with and without depressive syndromes of high-molecular weight precursor forms of neurohormones, which recent data suggest may be particularly abundant in and which support an active role for the CSF in neurohormonal communication within the brain.

Longitudinal Studies of Patients With Cushing's Disease

We propose studies of the longitudinal studies of CNS function in Cushing's disease patients before and after treatment to assess (1) the time course of recovery of central neurohormonal function in relation to the return of autonomous pituitary-adrenal function, and (2) the effects of treatment with drugs that (at least acutely) increase central CRH secretion (e.g. ipsapirone). One important question: are findings in rats of suppression of CRH mRNA following chronic treatment with imipramine and other antidepressant treatments (many of which increase pituitary-adrenal function after acute treatment) present even in pathological conditions in which CRH is already decreased, or is initially-low CRH actually increased to normal levels in these conditions? Although studies in Lewis rats will help address this question, it would also be important to examine in post-operative Cushing's patients as both a human analog of Lewis rats and also a human model of primary atypical depression. We shall also examine responsiveness of post-operative Cushing's patients to Type I and II glucocorticoid antagonists as proposed above and by Dr. Gold for atypical depression.

Immunologic Studies of Patients with Melancholia: Comparison with Atypical Depression

Studies of immunological function in patients with depression in which we will attempt to identify indices of immune function reflecting sustained hypo- vs. hypercortisolism, given the known data regarding the effects of glucocorticoids on lymphocyte numbers, subpopulations, and function. Hence, we hypothesize that in melancholia we will see evidence of immunosuppression due to glucocorticoid excess, which include reduced numbers of CD4-positive T-lymphocytes and which may account for some of the findings in the literature showing attenuated proliferative responses of peripheral blood lymphocytes (PBL) to mitogen stimulation. We will also utilize more recently-developed techniques of quantitating subpopulations of (PBL), such as two- and three-color FACS analysis that can now be used to identify a variety of cell surface markers on specific subgroups of immunocompetent cells. On the other hand, we would expect to find evidence of immune enhancement in patients with atypical depressions; preliminary data already suggest this is the case in patients with chronic fatigue syndrome, who show evidence of increased differentiation of CD4-positive lymphocytes which also bear the CD45 antigen. We will also study patients with adrenal insufficiency through our collaborations with NICHD to examine the effects of extreme hypocortisolism on these measures.

Procaine as a Probe of the Hypothalamic CRH Neuron

We plan continued studies of the effects of procaine in patients with affective illness, the first of which will be conducted in collaboration with Dr. Post, using 15O-H₂O PET scanning to image cerebral blood flow, in conjunction with measurement of ACTH and cortisol responses to this agent. These data may be useful in more directly correlating changes in brain physiology with endocrine responses. In addition, we plan to conduct studies of our own to better define the

characteristics of the ACTH response in patients with depressive syndromes and controls in a more standard endocrine challenge paradigm, in which different doses of procaine or placebo would be given on different evenings, in analogous fashion to our studies of responses to a variety of endocrine challenges, including CRH, ACTH and arginine vasopressin. We would also plan to examine the effects of procaine on peripheral catecholamine secretion using the arterial kinetics methodology outlined above, to determine whether or not the ACTH responses are associated with concordant changes in the peripheral secretion of NE and EPI; we shall also subsequently conduct pharmacologic blockade studies to determine if these responses are blocked by specific neurotransmitter antagonists, such as serotonergic or dopaminergic antagonists.

Role of the Type I GR in the Regulation of the Primate HPA Axis

The type II glucocorticoid receptor gene is among the best characterized of all mammalian genes. Its promoter has been isolated, sequenced and analyzed. Moreover, the post-translational phosphorylation phenomenon has been precisely mapped on the receptor protein and has been implicated as vital to its role as a transactivating factor. In addition, the functional domains of the protein for ligand binding, DNA binding, and its protein-protein interactions with heat shock proteins and fos-jun heterodimer have been characterized.

While the physiological role of the type II GR in the rat and primate has also been extensively studied, the role of the type I glucocorticoid receptor in the regulation of the rat and primate hypothalamic-pituitary-adrenal axis has not been fully elucidated. On the one hand, the majority of data clearly indicate that the principal role for the hippocampal type I glucocorticoid receptor in the regulation of the HPA axis is in restraining the PVN CRH neuron. Compatible with this idea is Dr. Brady's data showing that chronic tricyclic antidepressants and fluoxetine cause a significant decrease in the expression of CRH mRNA in the PVN in association with a significant increase in the expression of type I but not type II glucocorticoid receptor mRNA in the hippocampus. On the other hand, Dr. Brady has shown that selective destruction of hippocampal dentate gyrus granule cells by colchicine injection causes a decrease rather than an increase in the expression of PVN CRH mRNA. Moreover, Dr. Sternberg will present data showing that the number of type I glucocorticoid receptors is decreased rather than increased in LEW/N rats that show a consistent hyporesponsiveness of the PVN CRH neuron to a variety of inflammatory, neurotransmitter, and behavioral stimuli. These latter data indicate that glucocorticoid (at least type I GR) modulation of the CRH neuron is not necessarily strictly of an inhibitory nature, but that time-specific and site-specific factors must be introduced into the equation.

In the light of our demonstration that the type I GR gene is expressed primate hippocampus and the potential importance of the type I GR receptor to the themes of our basic and clinical research groups, we plan a series of studies aimed at studying molecular and physiological aspects of this receptor, as well as its potential involvement in pathophysiological states.

Structure/Function Studies on the Regulation of the Human Type I GR Gene:

We are currently screening human somatic genomic libraries to isolate clones encoding the MR gene with a specific emphasis on obtaining the promoter/enhancer regulatory elements lying upstream of the transcription start site. Once the putative positive clones are identified we shall re-ascertain the functional elements with sequencing and subsequent construction of

reporter gene chimera that will be transfected into an appropriate cell line to confirm the promoter/enhancer sequences driving reporter gene expression.

These fusion gene chimera will be used to screen the effect of a variety of antidepressant drugs, neurohormones, and other physiological modulators on reporter gene expression in transient and stable transfection assays. Any significantly responsive region will be further analyzed by creating nested deletions in the sequences, and the smallest responsive segment in the MR gene promoter will be characterized. The putative consensus cis-acting element within this domain will be further characterized for its interaction with transacting protein factors utilizing gel shift assays and DNA footprinting analysis.

Post-transcriptional Regulation of the type 1 GR:

We also wish to study aspects of post-translational modification-effects on the regulation of the type I receptor's function. It has recently been shown that other members of the steroid receptor superfamily (COUP and the progesterone receptor) can be activated to stimulate transcription of target promoter in vitro in the absence of endogenous steroid by compounds that mediate their actions through cell surface receptors. Kinases stimulated by second messenger systems have been implicated in these mechanisms because a ligand that mediated these actions, dopamine, utilizes receptors linked to kinase activity and that okadaic acid (a phosphatase inhibitor) can show the same activating properties.

We propose a series of experiments in which either the hMR expression plasmid or the hGR expression plasmid, along with a target promoter linked to a reporter gene, MMTV-CAT would be co-transfected into cells containing no endogenous level of the other type of receptor. A series of drugs, neurotransmitters and peptide hormones involved in the stress response would then be tested for their ability to stimulate expression of the target promoter. AVP, fragments of ACTH, and reserpine have been implicated in the activation of glucocorticoid receptors in a corticosteroid-independent fashion. It would be interesting to test these agents as well as others to see if ligand-independent transcription can be achieved and if this action is specific for one or the other receptor. Perhaps a mechanism of this type could explain some of the differential regulation of the two receptors. The presence of increased phosphorylation as a potential cause of any changes detected could be determined by 2D gel electrophoresis or HPLC peptide mapping.

In vivo Studies on the Relevance of the Type 1 GR to Primate HPA Function:

We plan to administer the glucocorticoid receptor antagonist RU 28318 or its active metabolite RU 26753 to chronically tethered, freely moving rhesus macaques at 8:00 PM when the inhibitory effect of GR type I is maximally exerted. We anticipated a dose-dependent activation of the HPA axis, a phenomenon that does not occur with the administration of the type II GR antagonist RU 38486 at that time of day because of the relative paucity of endogenous CRH. Because these receptors are primarily hippocampal, such a paradigm will also provide useful information regarding the function integrity of the suprahypothalamic component of the HPA axis see below.

We have filed for an IND for the type 1 receptor antagonist ZK 91587 which is a potent mineralocorticoid receptor antagonist devoid of antiandrogen effects that has been previously given safely to humans. We plan a three-day trial of this compound in volunteers to explore its effects on the 24-hour basal circadian pattern of plasma ACTH and cortisol secretion and to estimate its effects on endogenous CRH release by monitoring the pituitary-adrenal responses to arginine vasopressin. We have also obtained approval for a four-week trial of imipramine in

healthy volunteers to explore the impact of this agent on the human HPA axis. To determine if imipramine influences human HPA function by interacting with the type 1 GR, we plan to evaluate the effects of a three-day trial of ZK 91587 before and during imipramine treatment.

In vitro Studies of the Type 1 GR Receptor:

In the light of Dr. Brady's finding regarding the potential role of site specific (e.g. dentate gyrus) type 1 GR receptors in the regulation of the PVN CRH neuron, Dr. Brady will spearhead studies to explore the relative number type 1 and type II GR's and the expression of type 1 and type II GR mRNA's in specific hippocampal subfields under varying conditions. Dr. Sternberg has described her plans to collaborate with Dr. Brady on exploring these parameters in adrenalectomized and non-adrenalectomized LEW/N and F344/N rats, as well as possible studies in these species in whom selective hippocampal lesions have been made.

Studies of the Functional Integrity of Glucocorticoid Receptor Genes:

Studies of the structure-function relationships of the type II glucocorticoid receptor gene indicate that mutations in different sites of the gene (exclusive of the 5' regulatory region) could promote tissue-specific glucocorticoid resistance, including hippocampal or hypothalamic resistance. This premise rests on data that the glucocorticoid receptor not only contains regions for binding of steroid hormone and for binding to DNA in regulatory regions of target genes; recent data now show that other domains influence its capacity for phosphorylation (and hence activation without ligand) in the cytoplasm, its translocation to the nucleus, its capacity to bind to other ubiquitous transcription factors such as c-jun and c-fos, and its capacity to form dimers and heterodimers. In this regard, a mutation in a domain influencing a given mode of action may have effects in one tissue where this mode is important but leave glucocorticoid receptor actions intact in another. An example of this non-generalized steroid resistance is X-linked spinal and bulbar muscular atrophy (Kennedy's disease), an adult-onset neurological disease. This disease is associated with mutations of the androgen receptor, associated with enlargement of the CAG repeat in the amino terminal domain, but few, if any, signs of generalized androgen insensitivity. Elucidating potential mutations of glucocorticoid receptors associated with normal HPA axis activity is technically feasible, using PCR-GC-clamp assisted denaturing gel electrophoresis and/or the chemical or enzymatic mismatch method. These would provide suggestions as to whether there are mutations or deletions in the receptors of these patients and would prompt us to further proceed by sequencing their receptor gene. If such mutations are found, their function could be determined in co-transfection assays. Demonstration of dysfunction will confirm an alteration in the functional characteristics of the receptor, but a failure at such a demonstration would not exclude a pathophysiologically significant alteration, and different co-transfection bioassays, employing different glucocorticoid responsive promoters and/or different host cell, would be employed.

Development and/or Application of New Means of Assessing Functional Glucocorticoid Status :

The demonstration of functionally relevant hypocortisolism is difficult at best, and may be facilitated by the application of new stimulation paradigms outlined above. However, we need additional means of evaluating a patient's actual glucocorticoid status. It has been shown that in the syndrome of glucocorticoid-resistant asthma (which may reflect focal glucocorticoid resistance either in bronchi or immune cells), there is a concomitant glucocorticoid resistance in skin that can be quantified by assessing the vasodilatation produced by a careful application of a fixed dose of corticosteroid. These patients also show a relative failure to respond to dexamethasone-induced suppression of mitogen-induced transformation or production of

cytokines by short-term cultured leukocytes. Additional approaches include quantification of the recently cloned PLA activating factor mRNA encoding a protein that may be crucial to the way in which glucocorticoids exert their effects upon immune function. Accessibility of type I receptors is more problematic, though they are present in large quantities in rectal mucosa, can be obtained by biopsy, or functionally evaluated by evaluation of mineralocorticoid-induced ion flux.

Studies of Other Components of the Stress Response:

We have concentrated on developing strategies and methodologies for studying CRH and related stress-responsive neurohormones and neurotransmitters in the pathophysiology of psychiatric, endocrine, and inflammatory diseases. We are aware that our emphasis on the CRH and the LC-NE systems fails to take into account the role of other CNS elements, particularly the mesolimbic dopaminergic systems that are thought to play an important role in hedonic, motor, and arousal processes of relevance to the symptom complex of affective illness. In addition, our previous work has failed to explore the potential linkages between the CRH and dopaminergic systems that are suggested by recent data showing that CRH antisera attenuate the behavioral sensitization that occurs as a consequence of the repeated administration of dopamine agonists. In this regard, one of many lacuna in our clinical and basic work of the stress response and its role in depression is in the study of dopaminergic systems. Unfortunately, this is not an area with which I am very familiar, though Dr. Kling has focal clinical interests, including the potential role of dopamine in the endocrine, behavioral, and neurophysiological effects of local anaesthetics. Drs. Brady and Herkenham in our basic science group also have focal interests in dopaminergic regulation that represent a reasonable starting point for us; namely, studies in both rat and non-human primate of the effects of antidepressant agents on components of the mesolimbic and mesocortical dopaminergic systems. Drs. Brady and Herkenham also plan to study central dopaminergic systems in post-mortem brains taken from depressed individuals. However, the extent to which we can, and ought to, broaden our focus given current limitations in resources and expertise remains an open question.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02584 02 CNE
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Common Pathway...Atypical Depressive Syndromes in Cushing's, SAD, CFS, & Hypothy		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Co-PI: Philip W. Gold, M.D., Chief, Clinical Neuroendocrinology Branch, NIMH Co-PI: Mitchel A. Kling, M.D., Chief, Unit on Affective Disorders, Clinical Neuroendocrinology Branch, NIMH Others: Dr. L.S. Brady Senior Staff Fellow CNE, NIMH Dr. M.D. DeBellis PRAT Fellow - NIGMS CNE, NIMH Dr. G. Cizza Visiting Fellow CNE, NIMH Dr. M.A. Demitrack Director, Eating Disorders Program, Univ. of Michigan Medical Center, Ann Arbor, MI		
COOPERATING UNITS (if any)		(Continued on p. 2)
LAB/BRANCH Clinical Neuroendocrinology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS 8.5	PROFESSIONAL: 6	OTHER: 2.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Our studies comparing and contrasting the pathophysiology of the depression and hypercortisolism in melancholia and Cushing's disease show that hypercortisolism in the former is associated with <u>CRH hypersecretion</u>, while hypercortisolism in the latter is associated with suppression of the <u>PVN CRH neuron</u> as a consequence of long-standing pituitary-mediated hyperadrenalism. While clinical data show that melancholic depression reflects an organized state of hyperarousal and anxiety, our clinical data show that the depressive syndrome associated with Cushing's disease is almost universally an atypical depressive syndrome, associated with lethargy, fatigue, hypersomnia, and hyperphagia. Moreover, follow-up studies of post-operative Cushing's disease patients whose PVN CRH neurons can remain suppressed for several months following surgically-induced remission of hypercortisolism show that as CRH neuron function returns, patients begin manifesting evidence of severe melancholic depression. Studies of other medical syndromes associated with atypical depression also suggest a subtle central pituitary-adrenal insufficiency as a consequence of a PVN CRH deficiency. These include the <u>chronic fatigue syndrome</u>, <u>seasonal affective disorder</u>, and <u>hypothyroidism</u>. To further refine our capacity to detect a subtle central adrenal insufficiency, we have designed and validated a number of new stimuli for the CRH neuron, including the infusion of arginine vasopressin, methoxyamine, ipsapirone, procaine, and the application of <u>graded levels of treadmill exercise</u> based on VO2 max. We have tested a number of pharmacologic agents in experimental animals for their capacity to activate the hypothalamic CRH neuron on a chronic basis without tolerance development, and have identified <u>type 1 glucocorticoid receptor</u> antagonists as the most potent. In this regard, we have obtained the genomic clone of the human mineralocorticoid receptor and have sequenced large portions of its 5' regulatory region premonitory to studies exploring more direct ways to manipulate the expression and functional activity of this important regulatory component for the PVN CRH neuron. </p>		

Objectives: This project represents the second of our three principal efforts to elucidate the molecular and biochemical mechanisms of physical and emotional stress and their relevance to major psychiatric disorders. In contrast to our project on melancholia, whose principal aim was to explore the hypothesis that this form of depression represented an activation of the major effectors that had principally escaped their usual counter-regulation, the present project examines the hypothesis that the lethargy, hypersomnia, and hyperphagia of atypical depression represents an excessive counter-regulation of the generalized stress response. An immediate aim of this project is to define the molecular and biochemical bases the atypical depressions that occur across the boundaries of a variety of medical and psychiatric disorders, and to develop more rapid and specific therapeutic interventions based on neuropharmacologic modulation of the major effectors of the stress response. A long-term goal is to utilize the information gained regarding pathophysiological features of the various forms of atypical depression to focus on candidate genes whose dysregulation confers susceptibility to this illness. The work for this project is hypothesis driven, proceeds in parallel on the clinical research unit and in the basic laboratory, and requires the close collaboration and cooperation of neuroendocrinologists, psychiatrists, molecular biologists, neurobiologists, and neuropharmacologists.

Methods employed: A variety of techniques have been developed and/or are applied on the clinical research unit for the clinical study of patients with melancholic depression and controls, including methods for the assessment of basal metabolic rate, norepinephrine spillover rate into arterial plasma, circadian pattern of neurotransmitter and neuropeptide release into cerebrospinal fluid, assessment of the cortisol and ACTH production rates, and many paradigms for the assessment of the functional integrity of each component of the hypothalamic pituitary-adrenal, gonadal, and thyroid axes. In the laboratory, we have raised antisera to a variety of neuropeptides including ovine and rat/human CRH, ACTH and various fragments, beta-endorphin, atrial natriuretic factor, arginine vasopressin, oxytocin, dynorphin, tumor necrosis factor, interleukin 1, interleukin 2, interleukin 6, neuropeptide Y, neuropeptide YY, cholecystokinin, and met-enkephalin. These antisera are used for radioimmunoassay, immunohistochemistry, and immunoneutralization studies. Affinity purification of antibody and immunohistochemistry procedures for these peptides have also been established. We also utilize gel and HPLC chromatographic methods for purification and identification of ovine and rat/human CRH, and for POMC fragments. Specific peptide antagonists for CRH, arginine vasopressin receptor subtypes, oxytocin, and cholecystokinin are also utilized. Additional methodologies include high resolution autoradiography, an ACTH bioassay in which rat adrenal corticosterone is examined as an endpoint, dispersed anterior pituitary cell cultures for examination of CRH activity or various extrahypothalamic substances with CRH bioactivity but not immunoreactivity, and a hypothalamic organ culture system for the assessment of factors regulating the acute release of CRH, TRH, and arginine vasopressin. We also employ an intravenously cannulated rat preparation with chronic maintenance and a system for maintenance of chronic central venous catheters in both the rat and non-human primate. Chronic intraventricular cannulae are also maintained in operantly conditioned non-human primates for studies of the behavioral effects of centrally-administered neuropeptides. Molecular methodologies include *in situ* hybridization, Northern blotting, transient and stable transfections, and standard cloning and sequencing procedures applied to the study of the type I glucocorticoid receptor and enkephalin genes.

Project Description: The following sections will outline our principal hypotheses regarding pathophysiological mechanisms in the various forms of atypical depression, our clinical and basic data regarding our hypothetical models, the significance of this work to the biomedical program of the institute, and our future directions.

HYPOTHESIS: THE CLINICAL AND BIOCHEMICAL MANIFESTIONS OF ATYPICAL DEPRESSION (DEFINED BY THE PRESENCE OF HYPERPHAGIA AND HYPERSOMNIA) REFLECT A PATHOLOGICAL INACTIVATION OF THE CRH AND LC-NE SYSTEMS

CLINICAL DATA

Atypical Depression as a State of Hypoarousal: Clinical Evidence for Inhibition of Arousal Producing, Stress-Responsive Neurotransmitters in the Pathophysiology of Atypical Depression

In contrast to the intense hyperarousal characteristic of melancholic depression, the syndrome of atypical depression seems to represent an excessive counter-regulation of the generalized stress response. The facilitation of pathways subserving arousal and attention in melancholic depression is replaced with apathy, lethargy, and passivity in atypical depression. Similarly, the inhibition of pathways subserving vegetative functions in melancholia contrasts with the hyperphagia and hypersomnia that are among the defining characteristics of atypical depression.

Putative Pathophysiological Alterations in The Stress System in Various Forms of Atypical Depression

The syndrome of atypical depression accounts for approximately 15% of idiopathic major depressions and is the form of depression most commonly seen in bipolar depression and in seasonal affective disorder. This syndrome also occurs across the boundaries of a variety of medical illnesses including Cushing's disease, hypothyroidism, the Chronic Fatigue Syndrome, and rheumatoid arthritis.

Cushing's Disease: Though Cushing's disease and melancholia are both associated with hypercortisolism and depression, our data indicating suppression of the CRH system rather than its activation in Cushing's disease helped us to understand why the clinical manifestations of depression in Cushing's disease differed so from those seen in melancholia. Four lines of data allowed us to conclude that the CRH system was organized differently in melancholia and Cushing's disease. First, we showed that patients with Cushing's disease showed an exaggerated rather than a blunted ACTH response to ovine CRH despite profound hypercortisolism, indicating a gross defect in glucocorticoid feedback upon the pituitary. Second, post-operative patients with Cushing's disease, who frequently show adrenal insufficiency, manifested attenuated, but clear ACTH responses to exogenous CRH, indicating that their adrenal insufficiency reflected a CRH neuron suppressed by long-standing hypercortisolism rather than a pituitary corticotroph cell incapable of responding to endogenous CRH. Third, we showed that the ACTH response to exogenous ovine CRH in post-operative patients with Cushing's disease could be enhanced by priming with the pulsatile administration of synthetic human CRH given experimentally to reproduce the pattern of naturally occurring CRH pulsatile secretion. In this regard, it is a well established fact that pituitary hormonal responses to hypothalamic releasing factors require priming by prior exposure to the pulsatile release of these releasing factors. In additional support of the idea that the hypothalamic CRH neuron in Cushing's disease was suppressed by long-standing hypercortisolism, we showed that the levels of CRH in the CSF of patients with Cushing's disease were profoundly reduced..

Chronic Fatigue Syndrome: The chronic fatigue syndrome is defined by the Center for Disease Control (CDC) as an illness consisting of profound debilitating fatigue lasting six months or longer in the absence of any clearly definable systemic illness, and often associated with feverishness, myalgias, arthralgias, and high titers to a variety of viral antigens. In a study of 30 patients meeting CDC criteria for the Chronic Fatigue Syndrome (CFS) followed longitudinally for

over one year at the NIH Clinical Center in the laboratory of Dr. Steven Strauss, we showed that the lethargy and fatigue in patients with the CFS also seem to occur in the context of a hypofunctioning CRH neuron. Hence, we showed that despite a significant reduction in evening basal total and free cortisol levels and in 24 hour urinary free cortisol excretion, patients with the CFS showed blunted ACTH responses to ovine CRH. As in post-operative Cushing's disease patients, we surmise that the blunted ACTH response to CRH in the absence of hypercortisolism in patients with the CFS reflects a pituitary corticotroph cell insufficiently primed by endogenous CRH. In a study exploring the dose dependant responses of the adrenal cortex to five doses of synthetic ACTH, we also showed that patients with the CFS showed exaggerated cortisol responses to low doses of ACTH and blunted cortisol responses to high dose ACTH administration. These data suggest that in the context of a subtle central adrenal insufficiency, adrenocortical ACTH receptors have grown hyperresponsive to ACTH, but that because of an atrophy of the adrenal cortex because of a central CRH deficiency the adrenocortical response to high doses of ACTH is attenuated. We have previously seen this pattern of response in patients receiving alternate day glucocorticoid treatment and known to have a partial central adrenal insufficiency on this account.

Seasonal Affective Disorder: Patients with seasonal affective disorder whose depressions are classically associated with hyperphagia and hypersomnia show normal 24-hour basal circadian corticosteroids secretion but a significant attenuation and delay in the ACTH response to ovine CRH. This finding suggestive of a subtle central CRH deficiency resolves after successful treatment of this syndrome with light therapy.

Hypothyroidism: Similarly, patients with hypothyroidism, who can present with symptoms of atypical depression, show responses to ovine CRH compatible with central adrenal insufficiency, consisting of exaggerated, delayed ACTH responses to CRH in association with a decreased cortisol response to the ACTH released during the course of the CRH stimulation test. In hypothyroid rats, we have shown a significant reduction in hypothalamic CRH content and mRNA in association with an increase in hippocampal glucocorticoid receptor number (see pre-clinical studies, below).

Rheumatoid Arthritis: In a subsequent section, we shall outline in detail Dr. Sternberg's work showing that hypofunctioning of the hypothalamic CRH neuron in the LEW/N rat is associated with both susceptibility to inflammatory disease and abnormal behavioral responses to environmental stressors than can be construed to reflect the hypoarousal expected in a state of CRH deficiency. In this regard, in addition to their susceptibility to inflammatory disease, patients with rheumatoid arthritis also show a very high incidence of depression that is most commonly of the atypical variety. In fact, atypical depression-like symptoms are so common in rheumatoid arthritis that one of the major diagnostic instruments used for the diagnosis of affective disorders excludes the diagnosis of an affective disorder in patients with rheumatoid arthritis but not in patients with other debilitating chronic, relapsing disorders. We speculate that a neurobiologic abnormality resulting in excessive counter-regulation of the stress response may be associated with susceptibility to syndromes characterized by both inflammatory disease and atypical depression.

Excessive counter-regulation of the stress response as a factor predisposing to inflammatory/affective diseases raises the theoretical possibility of utilizing neuropharmacologic agents in the treatment of illnesses such as rheumatoid arthritis. As an example, we have recently shown that serotonin_{1A} agonists activate the pituitary-adrenal axis via a CRH-specific mechanism, raising the possibility of using new experimental serotonin_{1A} agonists such as ipsapirone in the treatment of inflammatory disease. Alternatively, one could employ agents that antagonize tonic inhibitory influences upon the CRH neuron like naloxone, an intervention that would theoretically

enhance the acute CRH response to inflammatory mediators. Finally, one should consider the theoretical possibility that drugs like the benzodiazepine alprazolam, that are among the most widely prescribed drugs in the world, influence the immunologic response by their gabamimetic effects. In this regard, we have shown that alprazolam inhibits the pituitary-adrenal axis in a dose dependant fashion by inhibiting CRH release, an effect that would be expected to potentially enhance the immunologic response and result in either increased susceptibility to inflammatory disease or resistance to infectious illness.

PRE-CLINICAL STUDIES

Effects of "Activating" Antidepressants preferentially effective in the treatment of atypical depression on the expression of genes encoding the principal effectors of the stress response

In contrast to the tricyclic antidepressants, which show some efficacy in the treatment of atypical depression, agents such as the MAO inhibitor phenelzine, the serotonin uptake inhibitor fluoxetine, and the experimental alpha-2 receptor antagonist idazoxan each seem somewhat more effective in the treatment of atypical depression. To explore whether this preferential efficacy reflected a differential effect on the expression of genes encoding proteins that played a key role in the stress response, we examined the effects of the acute and chronic administration of these agents on the expression of CRH mRNA in the PVN, TH mRNA in the LC, and of type 1 and type 2 glucocorticoid receptor mRNA in the hippocampus. In contrast to imipramine, each of these activating antidepressants caused a significant increase in TH mRNA expression in the LC after chronic but not acute administration. On the other hand, each of these agents caused a decrease in CRH mRNA expression in the PVN after chronic but not acute administration, usually in association with an increase in either type 1 or type 2 glucocorticoid receptor mRNA expression in the hippocampus and an inhibition of pituitary-adrenal function. These data suggest that an increase in TH mRNA expression following the chronic administration of antidepressant agents could be useful in screening agents for their potential efficacy in the treatment of atypical depression, and that a putative activation of LC-NE function may be an important element in the therapeutic efficacy of these agent (though we cannot draw strict inferences from TH mRNA expression in the LC to LC functional activity). The effects of these agents on the expression of CRH mRNA in the PVN are antithetical to those we would have predicted. These findings suggest the possibility that activation of the CRH neuron is an inherent part of every depression and a decrease in the expression in CRH mRNA expression a common factor in the mechanism of action of a variety of antidepressant agents. Alternatively, these data could suggest that the state of arousal of the experimental animal may play a role in the effects of psychotropic agents on PVN CRH mRNA expression. Accordingly, an activated or stressed organism might show a decrease in the expression of CRH mRNA in the PVN, while a hypoaroused, inactivated organism might show an increase. In this regard, the outbred rats used in the present experiment were presumably somewhat stressed rather than hyperaroused. In the light of the fact that tricyclics can at times treat both melancholic and atypical depression, we are pursuing the possibility that these agents as well as the activating antidepressants may cause an increase in CRH mRNA expression in the PVN in hypoaroused organisms, thus working to re-equilibrate a dysregulated stress response. To test this hypothesis we are now giving a variety of antidepressants, including tricyclics, MAO inhibitors, and fluoxetine, to both Lewis and Fisher rats, histocompatible strains whom Dr. Sternberg has shown have hypo- and hyper-responsive CRH neurons, respectively (see section on CNS factors in inflammatory diseases, below).

Effects of experimentally-induced hypo-and hyperthyroidism on hypothalamic-pituitary-adrenal function: Implications for the role of thyroid abnormalities in the susceptibility to major depression and its clinical presentation

We have conducted a series of *in vivo* and *in vitro* studies in the rat exploring the effects of varying intervals of experimentally-induced hypo-and hyperthyroidism on the functional integrity of each component of the hypothalamic-pituitary-adrenal axis. Our *in vivo* data show that hypothyroidism produced evidence of a subtle adrenal insufficiency, reflected in many parameters, including significant decreases in CSF corticosterone concentrations, decreased adrenal weights, and decreased responsiveness of the adrenal cortex to a variety of stimuli. *In vivo* evaluation of the hypothalamic component of the adrenal axis revealed exaggerated plasma ACTH responses to both hypoglycemic stress and IL-1 administration, but decreased corticosterone responses to the ACTH released during the application of these centrally-acting stimuli; *in vitro* evaluation of the hypothalamic component revealed decreased CRH mRNA expression under basal and stressed conditions, decreased CRH content under basal conditions, and decreased CRH release from hypothalamic organ culture following the application of either KCL or arginine vasopressin. Moreover, our data also revealed an increase in the number of type II glucocorticoid receptors in the hippocampus thought to play an important role in mediating the negative feedback effects of glucocorticoids upon the hypothalamic CRH neuron. *In vivo* evaluation of the pituitary corticotroph cell revealed a significant attenuation in plasma ACTH responses to arginine vasopressin administration; *in vitro* evaluation of this component of the axis revealed a decrease in anterior pituitary POMC mRNA expression and content, an increased responsiveness of dispersed anterior pituitary cells to CRH, and an increase in the number of anterior pituitary CRH receptors. *In vivo* evaluation of the adrenal cortex showed a significant reduction in the corticosterone response to ACTH administration following pre-treatment with dexamethasone and a significant decrease in the corticosterone release from cultured adrenals following incubation with ACTH. Taken together, our data suggest that experimentally-induced hypothyroidism is associated with a subtle adrenal insufficiency which is at least partially due to a central CRH deficiency. Hence, there were subtle, but significant increases in the ACTH responses to centrally-acting stimuli which we interpret to reflect both a disinhibition of the pituitary-corticotroph cell by basal hypocortisolism and an increase in the responsiveness of the anterior pituitary to exogenous CRH. Our *in vitro* data showing a decrease in the CRH mRNA expression during basal and stressed conditions support this formulation. The decreased *in vivo* ACTH response to arginine vasopressin infusion is also compatible with this formulation, because vasopressin-induced ACTH release is both independent of glucocorticoid negative feedback and depends upon an adequate supply of endogenous CRH. Moreover, the decrease in anterior pituitary POMC mRNA expression and content, as well as the increase in CRH receptor number and the responsiveness of anterior pituitary cells to synthetic CRH is also suggestive of a subtle central CRH deficiency. From a clinical perspective, these lines of evidence suggesting that hypothyroidism is associated with a concomitant central CRH deficiency are compatible with the observation that hypothyroidism is associated with a very high incidence of atypical depression. Moreover, these data indicate that the suppression of the CRH neuron in the various subtypes of atypical depression associated with different illnesses can occur through a variety of mechanisms, ranging from a long-term suppression of the CRH neuron by glucocorticoids in Cushing's disease to a possible thyroid-deficient association in enhanced hippocampal glucocorticoid receptor mediated feedback of the CRH neuron.

Previous studies suggesting that hyperthyroidism is associated with hyperadrenalism have neither definitively confirmed this point, evaluated the effects of the duration of hyperthyroidism on hypothalamic-pituitary-adrenal function, nor evaluated the site in the HPA axis that is principally affected. To address these questions, we investigated the functional integrity of each component of the HPA axis in sham-thyroidectomized rats at 7 or 60 days. Our *in vivo* and *in vitro* data provide compelling evidence of the presence of a central, CRH-mediated hypercortisolism in the context of hyperthyroidism. These data strongly suggest of a centrally-mediated hypercortisolism in

experimentally-induced hyperthyroidism are compatible with data that depression in patients with hyperthyroidism is often of the melancholic rather than atypical variety, and indirectly supports our hypothetical models regarding the role of stress-responsive, arousal producing transmitters like CRH in the pathophysiology of the melancholic and atypical depression.

An animal model of atypical depression in the primate

For the past two years, we have maintained a colony of fifty marmosets in Building 14 under the supervision of full-time Guest Worker Elizabeth Johnson-McClure. Dr. Johnson-McClure has had extensive experience with this species *Callitrix Jacchus*) and has been able to successfully breed them on campus, in contrast to efforts previously undertaken in Poolesville. Dr. Johnson-McClure has also developed a close relationship with these marmosets so that she is able to hold them in her arms and take blood for hormonal measurement in about thirty seconds without restraining them.

In the past two years, Dr. Johnson-McClure has validated scales to rate young marmoset mother-child interactions based on their positive-negative interactions (e.g., carrying, social exploration, nursing, food sharing, proximity huddling, vs. bite, grabbing, rejection, attempt rejection, etc.). Dr. Johnson-McClure has been able to show that a quotient representing the sum of caring behaviors minus the punitive behaviors correlates positively with head circumference, limb length, and weight at six months. Follow-up of these marmosets at puberty shows that those who had relatively unsatisfactory interactions with the mother continue to show subtle but significant signs of growth retardation.

In subsequent studies, Dr. Johnson-McClure has begun to study the response of those marmosets whom she has followed since birth to various behavioral situations. Having had extensive experience in evaluating the behavior of these pair-bonding primates, Dr. Johnson-McClure studied them under several social conditions which are by definition differentially stressful. These conditions are in order of stressfulness, least to most: family, heterozygous pairs, social isolation, and peer group pairs (three males and three females). The following observations have been made. Wasting, a serious syndrome associated with behavioral depression and anorexia, occurs most often in female subordinates in the peer group. Isolated and subordinate females show evidence of an inactivation of the CRH system (analogous, possibly to atypical depression) with decreased basal ACTH and cortisol levels and abnormal ACTH responses to ovine CRH. Behaviors in isolation and in subordinates are very similar, often occurring in short spurts and characterized by many infantile behaviors like infant crying.

In addition to further behavioral and physiological studies (the colony is continually increasing) of deepening complexity, as numbers permit we will hopefully be able to draw inferences about the vulnerability to development of behavioral depression or wasting in the peer groups or isolates based on mother-child interactions as potential influential parameters. We also hope to determine whether the development of subordination is related to the tenor of mother-child interactions. In the 10% of animals who develop the wasting syndrome, we plan an intensive investigation of brain function early on before profound weight loss has occurred (once the subtlest signs of wasting occur, the syndrome is invariably fatal, though ECT reversible); these studies include integration of the various techniques available in our preclinical laboratory including high resolution autoradiography for receptor mapping, as well as assessment of gene expression for a variety of neuropeptides and other proteins of interest utilizing *in situ* hybridization. Ultimately, if the model develops sufficiently so that there are large numbers and families with inherent vulnerabilities to mother-child maladaptation or wasting, other molecular techniques including screening for RFLP's may be feasible.

An animal model of atypical depression in the rat

We have developed an animal model of depression in the rat similar to that described above in the primate. Hence, Dr. Linda Brady has shown that in a stable peer group consisting of five Long-Evans rats (3 male and two female), that the most subordinate male develops a syndrome characterized by social withdrawal, inactivity, and wasting. Evaluation of hypothalamic-pituitary-adrenal function reveals clear evidence of hypocortisolism. Studies of CRH gene expression in the PVN of the hypothalamus show significantly decreased CRH mRNA content in this locus in subordinate animals. These data, in combination with our primate data, suggest that in certain conditions of chronic social stress, the generalized stress response becomes inactivated rather than activated, and that a behavioral syndrome that in some respects is morphologically similar to atypical depression develops.

Significance to the biomedical research of the program:

These studies take one step further our hypothetical model suggesting that dysfunction in the regulation in the stress response results in clinically-apparent disease. Our first data regarded pathological mechanism in melancholia, which we postulated reflected an acute generalized stress response that had escaped its usual counterregulation. We have now introduced and validated the concept that depressive illness associated with lethargy, fatigue, hypersomnia, and hyperphagia reflect a pathological inactivation of stress-responsive arousal producing informational substances. Implicit in this concept is that idea that under some circumstances, in vulnerable individuals, intensely stressful or chronically-stressful situations cause an inhibition rather than an activation of the generalized stress response, and that this perturbation has pathophysiological and symptomatic consequences. We have documented that these changes occur in a variety of medical illnesses associated with atypical depression syndromes, including Cushing's disease, the Chronic Fatigue Syndrome, hypothyroidism, seasonal affective disorder, and the late-luteal phased menstrual disorder. In this regard, our data suggest that like the anemias, a final common pathway defect reflecting distinctive pathophysiological processes may contribute to the clinical and biochemical manifestations of a common pathologic process that spans several illnesses. Current studies also are intensively investigating the possibility a deficient responsiveness of the CRH neuron confers susceptibility not only to atypical depression but to inflammatory diseases as well (see summary of project on inflammatory diseases). These concepts, if valid, provide a new metaphor for understanding the vicissitudes of the normal stress response, the pathophysiology of psychiatric disorders, and the susceptibility to inflammatory diseases, and pave the way for a deliberate effort to design novel drugs to activate the stress response as a means of treating, ameliorating, or preventing these illnesses. In this regard, our data showing that activating antidepressant agents particularly effective in the treatment of atypical depression preferentially increase the expression of the tyrosine hydroxylase gene in the locus ceruleus provide a clue regarding the specificity of these agents and suggest a possible means of screening additional agents for the treatment of this disorder.

Our studies exploring the relationship between experimental perturbations in thyroid function and the functional integrity of the hypothalamic-pituitary-adrenal axis provide the first clear data regarding the impact of hyper-and-hypothyroidism on the central regulation of the pituitary-adrenal axis. These data provide a further theoretical basis for the linkage of thyroid dysfunction and affective disorder, and are of possible relevance to behavioral perturbations that are a cardinal manifestation of thyroid disease. They also provide the theoretical and physiological basis for the clinical practice of adding glucocorticoid replacement to profoundly hypothyroid patients and to hypothyroid individuals prior to surgical stress.

Future Directions:

One of our principal goals in the coming year is to conduct an intensive series of studies in the basic laboratory to identify neuropharmacologic agents whose chronic administration produces a sustained activation of the hypothalamic CRH neuron and to identify the behavioral, physiological, and immunological consequences of this effect. Such agents will then be utilized in systematic efforts to treat the atypical depression associated with illnesses such as Cushing's disease, hypothyroidism, chronic fatigue syndrome, and seasonal affective disorder. These agents will also be applied in the treatment of inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease and the atypical depressions often associated with these illness (for more details, please see section on inflammatory diseases).

Our study of the pathophysiological mechanisms involved in the inactivation of the stress response depression will focus on exploring the factors that counter-regulate the stress response, with particular emphasis on the type I and type II glucocorticoid receptor genes. As noted in the section on melancholia, these genes represent the best studied in mammalian species, and structure function relationships indicate that specific focal mutations in the protein coding regions of these genes could produce tissue-specific abnormalities in their regulation of glucocorticoid-responsive genes. Hence, a focal abnormality in the hippocampus could confer the clinical and biochemical manifestations of atypical depression. As a corollary, we shall focus on delineating the role of the type I glucocorticoid receptor in the human and primate hypothalamic-pituitary-adrenal axis, in keeping with our demonstration that type I GR mRNA is abundantly expressed in primate hippocampus.

Clinically, we shall apply a series of protocols similar to those proposed in melancholia to explore pathophysiological mechanisms of hypocortisolism in atypical depression and to further clarify the nature of the defect in CRH secretion and that of other possible ACTH secretagogues. These studies will evaluate the functional integrity of each component of the hypothalamic-pituitary-adrenal axis. These include hypothalamic evaluation via sampling of the venous drainage of the anterior pituitary via inferior petrosal sinus sampling, assessment of the 24 hour circadian pattern of CRH and related informational substances into the CSF via continuous lumbar drainage for thirty hours, and assessment of the pituitary-adrenal responses to ipsapirone, a serotonin 1A agonist that we have shown produces pituitary-adrenal activation almost exclusively via activation of the hypothalamic CRH neuron. We have recently performed dose response studies at different times of day exploring the effects of intravenous arginine vasopressin infusion on plasma ACTH release. These studies were conducted because arginine vasopressin-induced ACTH release is not glucocorticoid suppressible, and because vasopressin-induced ACTH release is thought to principally reflect the amount of endogenous CRH in the hypophyseal portal blood. In this regard, vasopressin is a very weak secretagogue of ACTH itself, but potently synergizes with the effects of endogenous CRH. We also plan to evaluate the functional integrity of the pituitary corticotroph cell via the administration of synthetic CRH and to evaluate the dose dependant effects of synthetic ACTH on the functional integrity of the adrenal cortex. Studies are also underway to explore the expression of type I and type II GR mRNA on lymphocytes taken from patients with melancholia. An additional clinical focus in the coming year will attempt to further delineate glucocorticoid status in patients with melancholia by evaluating the expression of a variety of genes whose transcription rates are thought to be critically dependant on glucocorticoid modulation.

With the addition of Dr. Jay Joshi from Marshall Nirenberg's lab to our molecular biology laboratory, we now have the capacity to explore the potential role of viral proteins in the expression of neuropeptide genes such as enkephalin and CRH (see section on molecular biology). In the light of data that HTLV-II may be involved in some cases of the chronic fatigue syndrome, and Dr. Joshi's data that HTLV-II TAT protein markedly enhances the expression of the proenkephalin gene

(which in turn would suppress the release of CRH), we shall explore the possible implications of viral mechanisms in illnesses such as chronic fatigue syndrome.

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Demitrack MA, Gold PW, Dale JK, Krahn DD, Kling MA, Straus SE. Plasma and cerebrospinal fluid monoamine metabolism in patients with chronic fatigue syndrome: Preliminary Findings. 1992, *Biol Psychiatry*, in press.

Objectives: This project represents the second of our three principal efforts to elucidate the molecular and biochemical mechanisms of physical and emotional stress and their relevance to major psychiatric disorders. In contrast to our project on melancholia, whose principal aim was to explore the hypothesis that this form of depression represented an activation of the major effectors that had principally escaped their usual counter-regulation, the present project examines the hypothesis that the lethargy, hypersomnia, and hyperphagia of atypical depression represents an excessive counter-regulation of the generalized stress response. An immediate aim of this project is to define the molecular and biochemical bases the atypical depressions that occur across the boundaries of a variety of medical and psychiatric disorders, and to develop more rapid and specific therapeutic interventions based on neuropharmacologic modulation of the major effectors of the stress response. A long-term goal is to utilize the information gained regarding pathophysiological features of the various forms of atypical depression to focus on candidate genes whose dysregulation confers susceptibility to this illness. The work for this project is hypothesis driven, proceeds in parallel on the clinical research unit and in the basic laboratory, and requires the close collaboration and cooperation of neuroendocrinologists, psychiatrists, molecular biologists, neurobiologists, and neuropharmacologists.

Methods employed: A variety of techniques have been developed and/or are applied on the clinical research unit for the clinical study of patients with melancholic depression and controls, including methods for the assessment of basal metabolic rate, norepinephrine spillover rate into arterial plasma, circadian pattern of neurotransmitter and neuropeptide release into cerebrospinal fluid, assessment of the cortisol and ACTH production rates, and many paradigms for the assessment of the functional integrity of each component of the hypothalamic pituitary-adrenal, gonadal, and thyroid axes. In the laboratory, we have raised antisera to a variety of neuropeptides including ovine and rat/human CRH, ACTH and various fragments, beta-endorphin, atrial natriuretic factor, arginine vasopressin, oxytocin, dynorphin, tumor necrosis factor, interleukin 1, interleukin 2, interleukin 6, neuropeptide Y, neuropeptide YY, cholecystokinin, and met-enkephalin. These antisera are used for radioimmunoassay, immunohistochemistry, and immunoneutralization studies. Affinity purification of antibody and immunohistochemistry procedures for these peptides have also been established. We also utilize gel and HPLC chromatographic methods for purification and identification of ovine and rat/human CRH, and for POMC fragments. Specific peptide antagonists for CRH, arginine vasopressin receptor subtypes, oxytocin, and cholecystokinin are also utilized. Additional methodologies include high resolution autoradiography, an ACTH bioassay in which rat adrenal corticosterone is examined as an endpoint, dispersed anterior pituitary cell cultures for examination of CRH activity or various extrahypothalamic substances with CRH bioactivity but not immunoreactivity, and a hypothalamic organ culture system for the assessment of factors regulating the acute release of CRH, TRH, and arginine vasopressin. We also employ an intravenously cannulated rat preparation with chronic maintenance and a system for maintenance of chronic central venous catheters in both the rat and non-human primate. Chronic intraventricular cannulae are also maintained in operantly conditioned non-human primates for studies of the behavioral effects of centrally-administered neuropeptides. Molecular methodologies include in situ hybridization, Northern blotting, transient and stable transfections, and standard cloning and sequencing procedures applied to the study of the type I glucocorticoid receptor and enkephalin genes.

Project Description: The following sections will outline our principal hypotheses regarding pathophysiological mechanisms in the various forms of atypical depression, our clinical and basic data regarding our hypothetical models, the significance of this work to the biomedical program of the institute, and our future directions.

HYPOTHESIS: THE CLINICAL AND BIOCHEMICAL MANIFESTATIONS OF ATYPICAL DEPRESSION (DEFINED BY THE PRESENCE OF HYPERPHAGIA AND HYPERSONNIA) REFLECT A PATHOLOGICAL INACTIVATION OF THE CRH AND LC-NE SYSTEMS

CLINICAL DATA

Atypical Depression as a State of Hypoarousal: Clinical Evidence for Inhibition of Arousal Producing, Stress-Responsive Neurotransmitters in the Pathophysiology of Atypical Depression

In contrast to the intense hyperarousal characteristic of melancholic depression, the syndrome of atypical depression seems to represent an excessive counter-regulation of the generalized stress response. The facilitation of pathways subserving arousal and attention in melancholic depression is replaced with apathy, lethargy, and passivity in atypical depression. Similarly, the inhibition of pathways subserving vegetative functions in melancholia contrasts with the hyperphagia and hypersomnia that are among the defining characteristics of atypical depression.

Putative Pathophysiological Alterations in The Stress System in Various Forms of Atypical Depression

The syndrome of atypical depression accounts for approximately 15% of idiopathic major depressions and is the form of depression most commonly seen in bipolar depression and in seasonal affective disorder. This syndrome also occurs across the boundaries of a variety of medical illnesses including Cushing's disease, hypothyroidism, the Chronic Fatigue Syndrome, and rheumatoid arthritis.

Cushing's Disease: Though Cushing's disease and melancholia are both associated with hypercortisolism and depression, our data indicating suppression of the CRH system rather than its activation in Cushing's disease helped us to understand why the clinical manifestations of depression in Cushing's disease differed so from those seen in melancholia. Four lines of data allowed us to conclude that the CRH system was organized differently in melancholia and Cushing's disease. First, we showed that patients with Cushing's disease showed an exaggerated rather than a blunted ACTH response to ovine CRH despite profound hypercortisolism, indicating a gross defect in glucocorticoid feedback upon the pituitary. Second, post-operative patients with Cushing's disease, who frequently show adrenal insufficiency, manifested attenuated, but clear ACTH responses to exogenous CRH, indicating that their adrenal insufficiency reflected a CRH neuron suppressed by long-standing hypercortisolism rather than a pituitary corticotroph cell incapable of responding to endogenous CRH. Third, we showed that the ACTH response to exogenous ovine CRH in post-operative patients with Cushing's disease could be enhanced by priming with the pulsatile administration of synthetic human CRH given experimentally to reproduce the pattern of naturally occurring CRH pulsatile secretion. In this regard, it is a well established fact that pituitary hormonal responses to hypothalamic releasing factors require priming by prior exposure to the pulsatile release of these releasing factors. In additional support of the idea that the hypothalamic CRH neuron in Cushing's disease was suppressed by long-standing hypercortisolism, we showed that the levels of CRH in the CSF of patients with Cushing's disease were profoundly reduced..

Chronic Fatigue Syndrome: The chronic fatigue syndrome is defined by the Center for Disease Control (CDC) as an illness consisting of profound debilitating fatigue lasting six months or longer in the absence of any clearly definable systemic illness, and often associated with feverishness, myalgias, arthralgias, and high titers to a variety of viral antigens. In a study of 30 patients meeting CDC criteria for the Chronic Fatigue Syndrome (CFS) followed longitudinally for

over one year at the NIH Clinical Center in the laboratory of Dr. Steven Strauss, we showed that the lethargy and fatigue in patients with the CFS also seem to occur in the context of a hypofunctioning CRH neuron. Hence, we showed that despite a significant reduction in evening basal total and free cortisol levels and in 24 hour urinary free cortisol excretion, patients with the CFS showed blunted ACTH responses to ovine CRH. As in post-operative Cushing's disease patients, we surmise that the blunted ACTH response to CRH in the absence of hypercortisolism in patients with the CFS reflects a pituitary corticotroph cell insufficiently primed by endogenous CRH. In a study exploring the dose dependant responses of the adrenal cortex to five doses of synthetic ACTH, we also showed that patients with the CFS showed exaggerated cortisol responses to low doses of ACTH and blunted cortisol responses to high dose ACTH administration. These data suggest that in the context of a subtle central adrenal insufficiency, adrenocortical ACTH receptors have grown hyperresponsive to ACTH, but that because of an atrophy of the adrenal cortex because of a central CRH deficiency the adrenocortical response to high doses of ACTH is attenuated. We have previously seen this pattern of response in patients receiving alternate day glucocorticoid treatment and known to have a partial central adrenal insufficiency on this account.

Seasonal Affective Disorder: Patients with seasonal affective disorder whose depressions are classically associated with hyperphagia and hypersomnia show normal 24-hour basal circadian corticosteroids secretion but a significant attenuation and delay in the ACTH response to ovine CRH. This finding suggestive of a subtle central CRH deficiency resolves after successful treatment of this syndrome with light therapy.

Hypothyroidism: Similarly, patients with hypothyroidism, who can present with symptoms of atypical depression, show responses to ovine CRH compatible with central adrenal insufficiency, consisting of exaggerated, delayed ACTH responses to CRH in association with a decreased cortisol response to the ACTH released during the course of the CRH stimulation test. In hypothyroid rats, we have shown a significant reduction in hypothalamic CRH content and mRNA in association with an increase in hippocampal glucocorticoid receptor number (see pre-clinical studies, below).

Rheumatoid Arthritis: In a subsequent section, we shall outline in detail Dr. Sternberg's work showing that hypofunctioning of the hypothalamic CRH neuron in the LEW/N rat is associated with both susceptibility to inflammatory disease and abnormal behavioral responses to environmental stressors than can be construed to reflect the hypoarousal expected in a state of CRH deficiency. In this regard, in addition to their susceptibility to inflammatory disease, patients with rheumatoid arthritis also show a very high incidence of depression that is most commonly of the atypical variety. In fact, atypical depression-like symptoms are so common in rheumatoid arthritis that one of the major diagnostic instruments used for the diagnosis of affective disorders excludes the diagnosis of an affective disorder in patients with rheumatoid arthritis but not in patients with other debilitating chronic, relapsing disorders. We speculate that a neurobiologic abnormality resulting in excessive counter-regulation of the stress response may be associated with susceptibility to syndromes characterized by both inflammatory disease and atypical depression.

Excessive counter-regulation of the stress response as a factor predisposing to inflammatory/affective diseases raises the theoretical possibility of utilizing neuropharmacologic agents in the treatment of illnesses such as rheumatoid arthritis. As an example, we have recently shown that serotonin_{1A} agonists activate the pituitary-adrenal axis via a CRH-specific mechanism, raising the possibility of using new experimental serotonin_{1A} agonists such as ipsapirone in the treatment of inflammatory disease. Alternatively, one could employ agents that antagonize tonic inhibitory influences upon the CRH neuron like naloxone, an intervention that would theoretically

enhance the acute CRH response to inflammatory mediators. Finally, one should consider the theoretical possibility that drugs like the benzodiazepine alprazolam, that are among the most widely prescribed drugs in the world, influence the immunologic response by their gabamimetic effects. In this regard, we have shown that alprazolam inhibits the pituitary-adrenal axis in a dose dependant fashion by inhibiting CRH release, an effect that would be expected to potentially enhance the immunologic response and result in either increased susceptibility to inflammatory disease or resistance to infectious illness.

PRE-CLINICAL STUDIES

Effects of "Activating" Antidepressants preferentially effective in the treatment of atypical depression on the expression of genes encoding the principal effectors of the stress response

In contrast to the tricyclic antidepressants, which show some efficacy in the treatment of atypical depression, agents such as the MAO inhibitor phenelzine, the serotonin uptake inhibitor fluoxetine, and the experimental alpha-2 receptor antagonist idazoxan each seem somewhat more effective in the treatment of atypical depression. To explore whether this preferential efficacy reflected a differential effect on the expression of genes encoding proteins that played a key role in the stress response, we examined the effects of the acute and chronic administration of these agents on the expression of CRH mRNA in the PVN, TH mRNA in the LC, and of type 1 and type 2 glucocorticoid receptor mRNA in the hippocampus. In contrast to imipramine, each of these activating antidepressants caused a significant increase in TH mRNA expression in the LC after chronic but not acute administration. On the other hand, each of these agents caused a decrease in CRH mRNA expression in the PVN after chronic but not acute administration, usually in association with an increase in either type 1 or type 2 glucocorticoid receptor mRNA expression in the hippocampus and an inhibition of pituitary-adrenal function. These data suggest that an increase in TH mRNA expression following the chronic administration of antidepressant agents could be useful in screening agents for their potential efficacy in the treatment of atypical depression, and that a putative activation of LC-NE function may be an important element in the therapeutic efficacy of these agent (though we cannot draw strict inferences from TH mRNA expression in the LC to LC functional activity). The effects of these agents on the expression of CRH mRNA in the PVN are antithetical to those we would have predicted. These findings suggest the possibility that activation of the CRH neuron is an inherent part of every depression and a decrease in the expression in CRH mRNA expression a common factor in the mechanism of action of a variety of antidepressant agents. Alternatively, these data could suggest that the state of arousal of the experimental animal may play a role in the effects of psychotropic agents on PVN CRH mRNA expression. Accordingly, an activated or stressed organism might show a decrease in the expression of CRH mRNA in the PVN, while a hypoaroused, inactivated organism might show an increase. In this regard, the outbred rats used in the present experiment were presumably somewhat stressed rather than hyperaroused. In the light of the fact that tricyclics can at times treat both melancholic and atypical depression, we are pursuing the possibility that these agents as well as the activating antidepressants may cause an increase in CRH mRNA expression in the PVN in hypoaroused organisms, thus working to re-equilibrate a dysregulated stress response. To test this hypothesis we are now giving a variety of antidepressants, including tricyclics, MAO inhibitors, and fluoxetine, to both Lewis and Fisher rats, histocompatible strains whom Dr. Sternberg has shown have hypo- and hyper-responsive CRH neurons, respectively (see section on CNS factors in inflammatory diseases, below).

Effects of experimentally-induced hypo- and hyperthyroidism on hypothalamic-pituitary-adrenal function: Implications for the role of thyroid abnormalities in the susceptibility to major depression and its clinical presentation

We have conducted a series of *in vivo* and *in vitro* studies in the rat exploring the effects of varying intervals of experimentally-induced hypo- and hyperthyroidism on the functional integrity of each component of the hypothalamic-pituitary-adrenal axis. Our *in vivo* data show that hypothyroidism produced evidence of a subtle adrenal insufficiency, reflected in many parameters, including significant decreases in CSF corticosterone concentrations, decreased adrenal weights, and decreased responsiveness of the adrenal cortex to a variety of stimuli. *In vivo* evaluation of the hypothalamic component of the adrenal axis revealed exaggerated plasma ACTH responses to both hypoglycemic stress and IL-1 administration, but decreased corticosterone responses to the ACTH released during the application of these centrally-acting stimuli; *in vitro* evaluation of the hypothalamic component revealed decreased CRH mRNA expression under basal and stressed conditions, decreased CRH content under basal conditions, and decreased CRH release from hypothalamic organ culture following the application of either KCL or arginine vasopressin. Moreover, our data also revealed an increase in the number of type II glucocorticoid receptors in the hippocampus thought to play an important role in mediating the negative feedback effects of glucocorticoids upon the hypothalamic CRH neuron. *In vivo* evaluation of the pituitary corticotroph cell revealed a significant attenuation in plasma ACTH responses to arginine vasopressin administration; *in vitro* evaluation of this component of the axis revealed a decrease in anterior pituitary POMC mRNA expression and content, an increased responsiveness of dispersed anterior pituitary cells to CRH, and an increase in the number of anterior pituitary CRH receptors. *In vivo* evaluation of the adrenal cortex showed a significant reduction in the corticosterone response to ACTH administration following pre-treatment with dexamethasone and a significant decrease in the corticosterone release from cultured adrenals following incubation with ACTH. Taken together, our data suggest that experimentally-induced hypothyroidism is associated with a subtle adrenal insufficiency which is at least partially due to a central CRH deficiency. Hence, there were subtle, but significant increases in the ACTH responses to centrally-acting stimuli which we interpret to reflect both a disinhibition of the pituitary-corticotroph cell by basal hypocortisolism and an increase in the responsiveness of the anterior pituitary to exogenous CRH. Our *in vitro* data showing a decrease in the CRH mRNA expression during basal and stressed conditions support this formulation. The decreased *in vivo* ACTH response to arginine vasopressin infusion is also compatible with this formulation, because vasopressin-induced ACTH release is both independent of glucocorticoid negative feedback and depends upon an adequate supply of endogenous CRH. Moreover, the decrease in anterior pituitary POMC mRNA expression and content, as well as the increase in CRH receptor number and the responsiveness of anterior pituitary cells to synthetic CRH is also suggestive of a subtle central CRH deficiency. From a clinical perspective, these lines of evidence suggesting that hypothyroidism is associated with a concomitant central CRH deficiency are compatible with the observation that hypothyroidism is associated with a very high incidence of atypical depression. Moreover, these data indicate that the suppression of the CRH neuron in the various subtypes of atypical depression associated with different illnesses can occur through a variety of mechanisms, ranging from a long-term suppression of the CRH neuron by glucocorticoids in Cushing's disease to a possible thyroid-deficient association in enhanced hippocampal glucocorticoid receptor mediated feedback of the CRH neuron.

Previous studies suggesting that hyperthyroidism is associated with hyperadrenalism have neither definitively confirmed this point, evaluated the effects of the duration of hyperthyroidism on hypothalamic-pituitary-adrenal function, nor evaluated the site in the HPA axis that is principally affected. To address these questions, we investigated the functional integrity of each component of the HPA axis in sham-thyroidectomized rats at 7 or 60 days. Our *in vivo* and *in vitro* data provide compelling evidence of the presence of a central, CRH-mediated hypercortisolism in the context of hyperthyroidism. These data strongly suggestive of a centrally-mediated hypercortisolism in

experimentally-induced hyperthyroidism are compatible with data that depression in patients with hyperthyroidism is often of the melancholic rather than atypical variety, and indirectly supports our hypothetical models regarding the role of stress-responsive, arousal producing transmitters like CRH in the pathophysiology of the melancholic and atypical depression.

An animal model of atypical depression in the primate

For the past two years, we have maintained a colony of fifty marmosets in Building 14 under the supervision of full-time Guest Worker Elizabeth Johnson-McClure. Dr. Johnson-McClure has had extensive experience with this species *Callitrix jacchus* and has been able to successfully breed them on campus, in contrast to efforts previously undertaken in Poolesville. Dr. Johnson-McClure has also developed a close relationship with these marmosets so that she is able to hold them in her arms and take blood for hormonal measurement in about thirty seconds without restraining them.

In the past two years, Dr. Johnson-McClure has validated scales to rate young marmoset mother-child interactions based on their positive-negative interactions (e.g., carrying, social exploration, nursing, food sharing, proximity huddling, vs. bite, grabbing, rejection, attempt rejection, etc.). Dr. Johnson-McClure has been able to show that a quotient representing the sum of caring behaviors minus the punitive behaviors correlates positively with head circumference, limb length, and weight at six months. Follow-up of these marmosets at puberty shows that those who had relatively unsatisfactory interactions with the mother continue to show subtle but significant signs of growth retardation.

In subsequent studies, Dr. Johnson-McClure has begun to study the response of those marmosets whom she has followed since birth to various behavioral situations. Having had extensive experience in evaluating the behavior of these pair-bonding primates, Dr. Johnson-McClure studied them under several social conditions which are by definition differentially stressful. These conditions are in order of stressfulness, least to most: family, heterozygous pairs, social isolation, and peer group pairs (three males and three females). The following observations have been made. Wasting, a serious syndrome associated with behavioral depression and anorexia, occurs most often in female subordinates in the peer group. Isolated and subordinate females show evidence of an inactivation of the CRH system (analogous, possibly to atypical depression) with decreased basal ACTH and cortisol levels and abnormal ACTH responses to ovine CRH. Behaviors in isolation and in subordinates are very similar, often occurring in short spurts and characterized by many infantile behaviors like infant crying.

In addition to further behavioral and physiological studies (the colony is continually increasing) of deepening complexity, as numbers permit we will hopefully be able to draw inferences about the vulnerability to development of behavioral depression or wasting in the peer groups or isolates based on mother-child interactions as potential influential parameters. We also hope to determine whether the development of subordination is related to the tenor of mother-child interactions. In the 10% of animals who develop the wasting syndrome, we plan an intensive investigation of brain function early on before profound weight loss has occurred (once the subtlest signs of wasting occur, the syndrome is invariably fatal, though ECT reversible); these studies include integration of the various techniques available in our preclinical laboratory including high resolution autoradiography for receptor mapping, as well as assessment of gene expression for a variety of neuropeptides and other proteins of interest utilizing *in situ* hybridization. Ultimately, if the model develops sufficiently so that there are large numbers and families with inherent vulnerabilities to mother-child maladaptation or wasting, other molecular techniques including screening for RFLP's may be feasible.

An animal model of atypical depression in the rat

We have developed an animal model of depression in the rat similar to that described above in the primate. Hence, Dr. Linda Brady has shown that in a stable peer group consisting of five Long-Evans rats (3 male and two female), that the most subordinate male develops a syndrome characterized by social withdrawal, inactivity, and wasting. Evaluation of hypothalamic-pituitary-adrenal function reveals clear evidence of hypocortisolism. Studies of CRH gene expression in the PVN of the hypothalamus show significantly decreased CRH mRNA content in this locus in subordinate animals. These data, in combination with our primate data, suggest that in certain conditions of chronic social stress, the generalized stress response becomes inactivated rather than activated, and that a behavioral syndrome that in some respects is morphologically similar to atypical depression develops.

Significance to the biomedical research of the program:

These studies take one step further our hypothetical model suggesting that dysfunction in the regulation in the stress response results in clinically-apparent disease. Our first data regarded pathological mechanism in melancholia, which we postulated reflected an acute generalized stress response that had escaped its usual counterregulation. We have now introduced and validated the concept that depressive illness associated with lethargy, fatigue, hypersomnia, and hyperphagia reflect a pathological inactivation of stress-responsive arousal producing informational substances. Implicit in this concept is that idea that under some circumstances, in vulnerable individuals, intensely stressful or chronically-stressful situations cause an inhibition rather than an activation of the generalized stress response, and that this perturbation has pathophysiological and symptomatic consequences. We have documented that these changes occur in a variety of medical illnesses associated with atypical depression syndromes, including Cushing's disease, the Chronic Fatigue Syndrome, hypothyroidism, seasonal affective disorder, and the late-luteal phased menstrual disorder. In this regard, our data suggest that like the anemias, a final common pathway defect reflecting distinctive pathophysiological processes may contribute to the clinical and biochemical manifestations of a common pathologic process that spans several illnesses. Current studies also are intensively investigating the possibility a deficient responsiveness of the CRH neuron confers susceptibility not only to atypical depression but to inflammatory diseases as well (see summary of project on inflammatory diseases). These concepts, if valid, provide a new metaphor for understanding the vicissitudes of the normal stress response, the pathophysiology of psychiatric disorders, and the susceptibility to inflammatory diseases, and pave the way for a deliberate effort to design novel drugs to activate the stress response as a means of treating, ameliorating, or preventing these illnesses. In this regard, our data showing that activating antidepressant agents particularly effective in the treatment of atypical depression preferentially increase the expression of the tyrosine hydroxylase gene in the locus ceruleus provide a clue regarding the specificity of these agents and suggest a possible means of screening additional agents for the treatment of this disorder.

Our studies exploring the relationship between experimental perturbations in thyroid function and the functional integrity of the hypothalamic-pituitary-adrenal axis provide the first clear data regarding the impact of hyper-and-hypothyroidism on the central regulation of the pituitary-adrenal axis. These data provide a further theoretical basis for the linkage of thyroid dysfunction and affective disorder, and are of possible relevance to behavioral perturbations that are a cardinal manifestation of thyroid disease. They also provide the theoretical and physiological basis for the clinical practice of adding glucocorticoid replacement to profoundly hypothyroid patients and to hypothyroid individuals prior to surgical stress.

Future Directions:

One of our principal goals in the coming year is to conduct an intensive series of studies in the basic laboratory to identify neuropharmacologic agents whose chronic administration produces a sustained activation of the hypothalamic CRH neuron and to identify the behavioral, physiological, and immunological consequences of this effect. Such agents will then be utilized in systematic

efforts to treat the atypical depression associated with illnesses such as Cushing's disease, hypothyroidism, chronic fatigue syndrome, and seasonal affective disorder. These agents will also be applied in the treatment of inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease and the atypical depressions often associated with these illnesses (for more details, please see section on inflammatory diseases).

Our study of the pathophysiological mechanisms involved in the inactivation of the stress response depression will focus on exploring the factors that counter-regulate the stress response, with particular emphasis on the type I and type II glucocorticoid receptor genes. As noted in the section on melancholia, these genes represent the best studied in mammalian species, and structure function relationships indicate that specific focal mutations in the protein coding regions of these genes could produce tissue-specific abnormalities in their regulation of glucocorticoid-responsive genes. Hence, a focal abnormality in the hippocampus could confer the clinical and biochemical manifestations of atypical depression. As a corollary, we shall focus on delineating the role of the type I glucocorticoid receptor in the human and primate hypothalamic-pituitary-adrenal axis, in keeping with our demonstration that type I GR mRNA is abundantly expressed in primate hippocampus.

Clinically, we shall apply a series of protocols similar to those proposed in melancholia to explore pathophysiological mechanisms of hypocortisolism in atypical depression and to further clarify the nature of the defect in CRH secretion and that of other possible ACTH secretagogues. These studies will evaluate the functional integrity of each component of the hypothalamic-pituitary-adrenal axis. These include hypothalamic evaluation via sampling of the venous drainage of the anterior pituitary via inferior petrosal sinus sampling, assessment of the 24 hour circadian pattern of CRH and related informational substances into the CSF via continuous lumbar drainage for thirty hours, and assessment of the pituitary-adrenal responses to ipsapirone, a serotonin 1A agonist that we have shown produces pituitary-adrenal activation almost exclusively via activation of the hypothalamic CRH neuron. We have recently performed dose response studies at different times of day exploring the effects of intravenous arginine vasopressin infusion on plasma ACTH release. These studies were conducted because arginine vasopressin-induced ACTH release is not glucocorticoid suppressible, and because vasopressin-induced ACTH release is thought to principally reflect the amount of endogenous CRH in the hypophyseal portal blood. In this regard, vasopressin is a very weak secretagogue of ACTH itself, but potently synergizes with the effects of endogenous CRH. We also plan to evaluate the functional integrity of the pituitary corticotroph cell via the administration of synthetic CRH and to evaluate the dose dependent effects of synthetic ACTH on the functional integrity of the adrenal cortex. Studies are also underway to explore the expression of type I and type II GR mRNA on lymphocytes taken from patients with melancholia. An additional clinical focus in the coming year will attempt to further delineate glucocorticoid status in patients with melancholia by evaluating the expression of a variety of genes whose transcription rates are thought to be critically dependent on glucocorticoid modulation.

With the addition of Dr. Jay Joshi from Marshall Nirenberg's lab to our molecular biology laboratory, we now have the capacity to explore the potential role of viral proteins in the expression of neuropeptide genes such as enkephalin and CRH (see section on molecular biology). In the light of data that HTLV-II may be involved in some cases of the chronic fatigue syndrome, and Dr. Joshi's data that HTLV-II TAT protein markedly enhances the expression of the proenkephalin gene (which in turn would suppress the release of CRH), we shall explore the possible implications of viral mechanisms in illnesses such as chronic fatigue syndrome.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02585 02 CNE
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Role of the CNS in the Susceptibility to Inflammatory Illness		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Esther M. Sternberg, M.D., Chief, Unit on Neuroendocrine Immunology, Clinical Neuroendocrinology Branch, NIMH		
Others: Dr. P.W. Burnet Dr. J.B. Joshi Dr. S.T. Koutmos Dr. D. Michelson	Visiting Fellow Biologist Guest Researcher Guest Researcher	CNE, NIMH CNE, NIMH CNE, NIMH CNE, NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Neuroendocrinology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892		
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CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A negative feedback loop exists between the <u>immune</u> and <u>central nervous systems</u>, in which immune/pro-inflammatory mediators signal the <u>hypothalamic-pituitary-adrenal axis</u> to induce <u>glucocorticoid</u> mediated restraint of the immune response. We have established the physiologic significance of this feedback loop in our studies in <u>Lewis (LEW/N) rats</u>, in which we have shown that <u>susceptibility to inflammatory arthritis</u> is related to a defect in the central component of this negative feedback loop, resulting in deficient <u>CRH</u> responses to challenge with a variety of inflammatory and <u>stress mediators</u>. We now extend these initial studies to show that the LEW/N CRH hyporesponsiveness, relative to other strains, is profound, and extends across a variety of neurotransmitters and behavioral stressors. It appears as early in <u>ontogeny</u> as postnatal Day 14, and indicates that LEW/N rats fail to emerge from the stress-hyporesponsive period. It is associated with a variety of defined <u>behavioral patterns</u>, consistent with the differential HPA axis and neurotransmitter responsiveness of these two strains. Although several neurotransmitter systems do not differ in these strains (<u>norepinephrine</u>, <u>5HT2</u>, <u>acetylcholine</u>), the <u>benzodiazepine/GABA receptor complex</u>, <u>5-HT1A system</u>, and <u>glucocorticoid Type 1 and type 2 receptor systems</u> differ in receptor number and/or ligand metabolism in these strains. The differences are organ and/or CNS site specific, and may be secondary to or play a causal role in the CRH hyporesponsiveness, and could contribute to or amplify some of the behavioral and/or inflammatory disease phenotypes in these strains. Thus the LEW/N rat represents an example of a genetically based HPA axis hyporesponsiveness associated with susceptibility to inflammatory and behavioral disease. </p>		

(Continued from P. 1)

Principal Investigator:

Others:	Dr. B. Poltorak	Visiting Fellow	CNE, NIMH
	Mr. C.C. Smith	Chemist	CNE, NIMH
	Dr. E. Zelazowski	Professional Service	CNE, NIMH
		Contract	
	Dr. P. Zelazowski	Visiting Fellow	CNE, NIMH

Objectives:

(1) To determine the role of the HPA axis in susceptibility to inflammatory disease and its concordant role in behavioral patterns.

(2) To define the molecular mechanism of CRH hyporesponsiveness in relative inflammatory disease susceptible LEW/N and relatively inflammatory disease resistant F344/N rats.

Methods employed: A variety of techniques have been developed and/or are applied on the clinical research unit for the clinical study of patients with TRP EMS and other inflammatory diseases, and controls, including methods for the assessment of the circadian pattern of neurohormone, neurotransmitter and neuropeptide release into cerebrospinal fluid, assessment of the cortisol and ACTH production rates, and many paradigms for the assessment of the functional integrity of each component of the hypothalamic pituitary-adrenal, gonadal, and thyroid axes. In the laboratory, we have raised antisera to a variety of neuropeptides including ovine and rat/human CRH, ACTH and various fragments, beta-endorphin, atrial natriuretic factor, arginine vasopressin, oxytocin, dynorphin, tumor necrosis factor, interleukin 1, interleukin 2, interleukin 6, neuropeptide Y, neuropeptide YY, cholecystokinin, and met-enkephalin. These antisera are used for radioimmunoassay, immunohistochemistry, and immunoneutralization studies. Affinity purification of antibody and immunohistochemistry procedures for these peptides have also been established. We also utilize gel and HPLC chromatographic methods for purification and identification of ovine and rat/human CRH, and for POMC fragments. Specific peptide antagonists for CRH, arginine vasopressin receptor subtypes, oxytocin, and cholecystokinin are also utilized. Additional methodologies include high resolution autoradiography, an ACTH bioassay in which rat adrenal corticosterone is examined as an endpoint, dispersed anterior pituitary cell cultures for examination of ACTH activity or various extrahypothalamic substances with ACTH bioactivity but not immunoreactivity, and a hypothalamic organ culture system for the assessment of factors regulating the acute release of CRH, TRH, and arginine vasopressin. We also employ an intravenously cannulated rat preparation with chronic maintenance and a system for maintenance of chronic central venous catheters in both the rat and non-human primate. Chronic intravenous cannulae are also maintained in operantly conditioned non-human primates for studies of the behavioral effects of centrally-administered neuropeptides. Molecular methodologies include *in situ* hybridization, Northern blotting, transient and stable transfections, and standard cloning and sequencing procedures applied to the study of the type I glucocorticoid receptor and enkephalin genes. Behavioral methodologies include open field testing, acoustic and tactile startle response, swim and restraint studies. Pathology techniques include standard light microscopy, as well as special immunohistochemistry staining methods, and standard differential counts as well as FACS analysis of peripheral blood.

Background And Summary Of Research

A bidirectional communication occurs between immune mediators and central nervous system neurohormones that orchestrate the generalized stress response (see Fig. 1). Peripherally generated cytokines signal several neurohormonal systems in the brain, including hypothalamic corticotropin-releasing hormone (CRH), to participate in maintaining both immunologic and behavioral homeostasis. CRH not only counter-regulates inflammation through pituitary-adrenal activation and the anti-inflammatory action of glucocorticoids, but it also sets in motion a coordinated series of behavioral and physiological responses. Fig. 1. Bi-directional feedback loop between the immune system and the central nervous system.

A putative dysregulation in the hypothalamic-pituitary-adrenal response and this bidirectional communication, by genetic, infectious, toxic, or pharmacological means may confer susceptibility not only to inflammatory disease states characterized by both inflammatory and emotional disturbances may derive from coherent alterations in specific central nervous system pathways. Finally, it proposes that neuropharmacological modulation of the CNS components of this bidirectional communication may potentially be applied in the treatment of traditional inflammatory disorders.

We have established the physiologic significance of the immune system - central nervous system feedback loop in our initial studies in Lewis (LEW/N) and Fischer (F344/N) rats, in which we have shown that susceptibility to inflammatory arthritis is related to a defect in the central component of this negative feedback loop, resulting in deficient CRH responses challenge with a variety of inflammatory and stress mediators. We have now extend these studies to show that the LEW/N CRH hyporesponsiveness, relative to other stains, is profound, ad extend across a variety of neurotransmitters and behavioral stressors; it occurs as early in ontogeny as postnatal Day 14, and is associated with a variety of defined behavioral patterns, consistent with the differential HPA axis responsiveness. Thus, the LEW/N rat provides a genetic and developmental model for the analysis of the association between relative CRH hyporesponsiveness, behavioral responses to stress and susceptibility to inflammatory disease. Our work in progress in this area has focussed on the specific molecular and biochemical mechanisms of LEW/N CRH hyporesponsiveness, particularly regulation through the benzodiazepine/GABA, 5-HT1A and glucocorticoid Type 1 and type 2 receptor systems. We have also focused on further defining the behavioral responses of LEW/N and F344/N rats in order to identify a relatively non-invasive instrument to accurately characterize the behavioral phenotype of LEW/N x F344/N F1 and F2 offspring. This data will be used in genetic studies correlating the behavioral and HPA axis response phenotypes with the phenotype of susceptibility to inflammatory disease in order to determine whether these phenotypes are coordinately controlled by one or more genes.

Mechanisms Of Relative CRH Hypo-And Hyper-Responsiveness in LEW/N And F344/N Rats, And Their Relationship To Inflammatory And Behavioral Responses To Stress

Specific Aims:

- To define the molecular mechanisms of relative CRH hypo- and hyper-responsiveness in inflammatory disease susceptible LEW/N and inflammatory disease resistant F344/N rats.
- To determine the role of relative CRH hypo- and hyper-responsiveness in LEW/N and F344/N rats' differential behavioral responses to stress.
- To determine the genetic control of the co-ordinate expression of CRH responsiveness, inflammatory disease susceptibility, and behavioral responses to stress in LEW/N and F344/N parent rats for

Previous work and work in progress:

Since 1989, our work has focused on: (1) defining the biochemical, functional, neuroanatomical and molecular extent of the defect in LEW/N rats associated with their relative CRH hypo-responsiveness and susceptibility to inflammatory disease, with a focus on neurotransmitter systems known to regulate CRH; (2) defining the range of stimuli to which LEW/N rats express defective HPA axis responses (including behavioral stresses); defining LEW/N and F344/N rats behavioral responses to stress, and determining the relationship of these response patterns to the relative CRH responses of these two strains; (3) defining the genetic mode of transmission of the phenotypes of arthritis susceptibility and HPA axis function in LEW/N x F344/N F1 and F2 offspring, in conjunction with probing LEW/N and F344/N parent rats for

RFLP's of candidate genes; (4) defining the ontogeny of CRH expression, and inflammatory and CRH responses to stress in LEW/N and F344/N rats.

Biochemical, neuroendocrine and neuroanatomical extent of the central nervous system defect associated with susceptibility to arthritis and inflammatory disease in the LEW/N rat.

Following up on our original findings, our subsequent studies focused on elucidation of the biochemical, neuroendocrine and neuroanatomical extent of the central nervous system defect associated with susceptibility to arthritis and inflammatory disease in the LEW/N rat. Because our previous studies had indicated a defect in regulation of CRH biosynthesis and secretion, subsequent studies focussed on evaluation of CRH regulatory mechanisms. Specific neurotransmitter regulatory systems chosen for study were based on our own data regarding the regulation of CRH release, including stimulatory pathways such as serotonin (5-HT), norepinephrine (NE), and acetylcholine ACh), and inhibitory pathways such as GABA and the glucocorticoids (see fig. 2, below, and Dr. Philip Gold's section for more detail). In addition, since initial *in vivo* studies had shown depressed pituitary responses in LEW/N compared to F344/N rats, we also extended these studies to define the extent of the pituitary defect both *in vivo* and *in vitro*.

Evaluation of the range and specificity of stimuli to which LEW/N rats show CRH hyporesponsiveness: *in vivo* and *in vitro* studies

Hypothalamic CRH responses to neurotransmitters and neurohormones:

In *in vitro* hypothalamic explant as well as *in vivo* studies, we found that LEW/N rats, compared to F344/N rats exhibited profound defects in hypothalamic CRH secretion in response to the neurotransmitters NE, ACh, 5-HT and quipazine (5-HT agonist), and corresponding profound blunting of plasma ACTH and corticosterone in response to i.p. arecholine (muscarinic ACh agonist). Alpha adrenergic, serotonergic (5-HT₂) and muscarinic cholinergic binding affinity and receptor numbers (K_d and B_{max}) were identical in LEW/N rats and F344/N rats. From these studies, we concluded that the LEW/N rat exhibits a broad defect in CRH responses to a variety of neurotransmitters, and suggested that the defect in CRH regulation was at the level of a common regulatory pathway for all these stimuli. This could include second messenger systems such as cAMP or components in the cAMP pathway, or glucocorticoid-receptor related mechanisms. The latter are thought to mediate the principal counter-regulatory restraint upon the CRH neuron.

Pituitary ACTH responses to neurohormones and second messengers:

Since initial *in vivo* studies showed that LEW/N pituitary ACTH responses to exogenous corticotropin releasing hormone (CRH) were blunted compared to F344/N, we evaluated basal and stimulated pituitary cell and pituitary organ explant ACTH responses. We found that basal ACTH secretion and peak ACTH response to CRH are 50% lower in LEW/N than F344/N rats, probably secondary to diminished basal ACTH content, POMC mRNA, and a decreased number of corticotrophs, in pituitary cell cultures from LEW/N compared to F344/N rats. Forskolin and 8Br cAMP stimulated ACTH secretion in both strains in a parallel fashion. CRH-stimulated ACTH secretion in LEW/N compared to F344/N pituitary cell cultures was more sensitive to dexamethasone and to corticosterone suppression. The data support the possibility of an HPA axis defect in LEW/N rats at the pituitary level which could be secondary to prolonged understimulation by hypothalamic CRH, or could also partially be related to enhanced glucocorticoid feedback inhibition.

Receptor binding studies in LEW/N and F344/N rats: focus on glucocorticoid, benzodiazepine and serotonergic receptors:

Although several neurotransmitter systems do not differ in these strains (norepinephrine, 5HT₂, acetylcholine), we have identified three neurotransmitter systems important in regulation of

CRH which do differ in receptor number and/or ligand metabolism in these strains: the benzodiazepine/GABA receptor complex, 5-HT_{1A} system, and glucocorticoid Type 1 and type 2 receptor systems. The differences are organ and/or CNS site specific, and may be secondary to or play a causal role in LEW/N CRH hyporesponsiveness. However, regardless of their role in the mechanism of the relative CRH hyporesponsiveness of LEW/N and relative CRH hyperresponsiveness of F344/N rats, these neurotransmitter/neuroendocrine feedback differences could contribute to or amplify some of the behavioral and/or inflammatory disease phenotypes in these strains.

Our receptor binding studies in LEW/N and F344/N rats have focused on glucocorticoid, benzodiazepine and serotonergic 5-HT_{1A} receptor systems because, on the one hand, the glucocorticoid (type 1 and type 2) and the GABA/benzodiazepine receptor systems represent major negative regulatory influences on the CRH response, and on the other, the serotonergic 5-HT_{1A} system is a positive regulatory stimulus which is itself regulated by glucocorticoid feedback.

GABA/benzodiazepine receptor binding:

Since the GABA/benzodiazepine receptor complex is an important inhibitory modulator of CRH secretion and responsiveness to excitatory stimuli, we examined *in vitro* binding of [3H]flunitrazepam to hypothalamic membrane preparations from LEW/N and F344/N rats. LEW/N rats had significantly more hypothalamic benzodiazepine binding sites [B_{max}] than F344/N rats, but there were no differences in benzodiazepine binding affinities (K_d) between these two strains. The differences in benzodiazepine receptor number were consistent with the respective plasma corticosterone levels in the two strains, and with previous reports indicating a negative correlation between corticosterone levels and benzodiazepine binding site number. Adrenalectomy of F344/N rats increased benzodiazepine binding to levels comparable to LEW/N animals, while adrenalectomy of LEW/N rats produced no alteration in receptor number. These findings suggest that basal benzodiazepine receptor differences between these strains may be secondary to strain differences in corticosterone levels. Since benzodiazepines attenuate hypothalamic CRH secretion through GABAergic inhibition, we suggest that strain differences in receptor number could also augment strain differences in hypothalamic-pituitary-adrenal (HPA) axis function through differential sensitivity to GABA-mediated feedback.

5-HT_{1A} receptor binding:

We also investigated the density of the 5-HT_{1A} receptor as well as the relative abundance of the receptor mRNA in various brain regions of LEW/N, outbred Harlan Sprague Dawley (HSD) and F344/N rats. In addition we have investigated the levels of 5-HT and its metabolite 5-hydroxyindole acetic acid (5-HIAA) in the same brain areas from these three strains of rat. LEW/N rats expressed significantly less hippocampal and frontal cortical 5-HT_{1A} binding sites and 5-HT_{1A} mRNA than HSD and F344/N rats. Similarly, levels of 5-HT in the hippocampus LEW/N were lower than in F344/N, while midbrain 5-HT levels in LEW/N rats was significantly lower than in either F344/N or HSD rats. All three strains showed a similar adrenalectomy-induced increase in hypothalamic 5-HIAA levels, the number of hippocampal 5-HT_{1A} binding sites, and hippocampal 5-HT_{1A} receptor mRNA expression. We conclude that the activity of some components of the central serotonergic system (5-HT_{1A} receptors and 5-HT turnover) in the cortex, hippocampus of the LEW/N rat is decreased compared to the F344/N rat and that this activity parallels the activity of their HPA axis.

Type 1 mineralocorticoid and Type 2 glucocorticoid receptor binding and mRNA expression:

To explore whether functional alterations in either the type I or type II GR's are potentially involved in the unresponsiveness of the PVNCRH neuron to a variety of stimuli, we examined their receptor binding in both hippocampus and hypothalamus, as well as in a variety of peripheral sites (pituitary, thymus and spleen). These studies were conducted in both adrenalectomized and

non-adrenalectomized LEW/N, F344/N and HSD rats. Preliminary studies in cytosolic extracts from adrenalectomized LEW/N and F344/N rats indicate significantly fewer hippocampal Type 1 and significantly more thymic Type 2 glucocorticoid receptors in LEW/N compared to F344/N rats.

We cannot definitively account for the finding of a significant decrease in type 1 glucocorticoid receptors in a species with a hyporesponsive CRH neuron. Such a finding could reflect a compensatory effort to restrict glucocorticoid negative feedback upon a CRH neuron pathologically suppressed by other factors. On the other hand, De Kloet has suggested that a critical factor in CRH regulation in a given tissue or brain region may be the ratio of Type 1 mineralocorticoid to Type 2 glucocorticoid receptors, rather than the absolute numbers of either receptor type. Thus, in LEW/N rats, with fewer hippocampal Type 1, but equal hippocampal Type 2 receptors, the overall ratio of hippocampal Type2/Type 1 is greater than in F344/N. Since Type 2 GR plays a critical role in suppression of stress response levels (as opposed to basal) of HPA axis hormones including CRH, a greater Type2/Type 1 ratio in LEW/N rats could explain their blunted HPA axis/CRH responses to a wide range of stressors. Finally, to the extent that a finding of a decrease in Type 1 receptors in the hippocampus of adrenalectomized LEW/N rats seems incompatible with the hyporesponsiveness of their CRH neuron, it should be noted that Dr. Brady in our group has found that colchicine-induced destruction of hippocampal dentate gyrus granular cells that contain Type 1 GR receptors results in a decrease rather than an increase in CRH mRNA, suggesting that not all Type 1 hippocampal GR receptors under all circumstances restrain the PVN CRH neuron.

Whether the finding of significantly more thymic Type 2 receptors in LEW/N compared to F344/N rats reflects a compensatory alteration to counter their glucocorticoid deficiency remains to be determined. If compensatory, such changes are clearly not sufficient to circumvent the consequences of the hyporesponsiveness of the CRH neuron and pituitary-adrenal axis in LEW/N rats.

Behavioral characteristics of SCW-arthritis susceptible LEW/N rats and SCW-arthritis resistant F344/N rats:

We also explored whether the relative hypoactivity and hyperactivity of the CRH neuron in LEW/N and F344/N rats were associated with behavioral differences in these histocompatible strains. These studies were undertaken in the light of data that CRH was not only a principal stimulus to the pituitary-adrenal axis, but also was capable of setting into motion a concerted series of other physiological as well as behavioral changes which have been construed as adaptive during stressful situations. Hence, the central administration of CRH to the rat activates the sympathetic nervous system, facilitates pathways that subserve arousal and cautious avoidance, and restrains pathways subserving program for growth and reproduction. In the course of these studies, we have found behavioral differences as well as differences in neuroendocrine responses to behavioral stressors in LEW/N and F344/N rats, consistent with their relative hypo- and hyper-CRH responsiveness: LEW/N rats exposed to a variety of behavioral stresses, including swim stress, restraint or ether stress exhibit profoundly blunted plasma ACTH and corticosterone responses to these stresses compared to F344/N rats. In open field studies LEW/N rats show greater exploratory behavior, consistent with a diminished CRH behavioral effect. Preliminary behavioral assessment of LEW/N and F344/N rats after intracerebroventricular (i.c.v.) administration of CRH indicate that LEW/N rats are more sensitive to the behavioral effects of low dose CRH than were F344/N rats. The latter finding is consistent with chronic under-secretion of CRH in LEW/N rats. Preliminary studies also indicate that these strains also differ dramatically in response to acoustic and tactile startle.

These studies thus show that these two strains differ significantly in several behavioral paradigms which reflect in part activity of the HPA axis. They suggest that the CRH neuron as a central nervous system element responding to peripherally-generated inflammatory mediators may play a dual role in the rat during the stress of injury or inflammation. The first of these is an immunologic role, in recruiting the pituitary-adrenal axis counter-regulation of the immune response, with the effect of preventing it from over-shooting; the second is a behavioral role, in promoting the kind of cautious avoidance that can be construed as adaptive in the context of acute injury or inflammatory process.

Genetic studies of arthritis phenotype in LEW/N and F344/N rats:

These studies, performed in collaboration with Dr. Ronald Wilder of NIAMS, indicate that LEW/N and F344/N parental strains exhibit a wide quantitative separation of the arthritic phenotype, (acute SCW arthritis) while F1 hybrids develop arthritis of intermediate severity. The distribution of the arthritic phenotype in the F2 generation was consistent with the hypothesis that genetic control of this quantitative trait was determined by a very limited number of genetic loci (quantitative trait loci calculated by Wright's method = 1.11). Because the major histocompatibility locus (MHC) is generally accepted as an important genetic determinant of autoimmune disease, restriction-fragment length polymorphisms within the rat MHC locus were studied for linkage to the acute SCW arthritis phenotype in the F2 intercross generation, and no linkage was observed.

Ontogeny of HPA axis responses and CRH gene expression in LEW/N and F344/N rats: LEW/N rats fail to emerge from the stress nonresponsive period:

In order to define the dynamics of the LEW/N CRH hypo-responsiveness over time, we investigated HPA axis responses to SCW during the post-natal developmental period in LEW/N and F344/N rats. We found that SCW-induced plasma corticosterone (CORT) responses do not significantly increase during development in LEW/N, while such responses clearly appear at postnatal day 14 in F344/N and outbred Harlan-Sprague-Dawley (HSD) rats. Additionally,

LEW/N rats fail to exhibit the normal ontogenic increase in CRH mRNA levels in the paraventricular nucleus (PVN), whereas their SCW-induced PVN CRH mRNA responses are blunted compared to F344/N at postnatal day 14. Taken together, these results suggest that LEW/N rats fail to emerge completely from their stress non-responsive period. This may account for the lack of stress responsiveness in young adult LEW/N rats, and, consequently, for their susceptibility to SCW-induced arthritis and other inflammatory diseases. These data indicate that the CRH regulatory defect in LEW/N rats is present early in life, and could potentially reflect a developmental defect involving the abnormal persistence of the expression of a gene in the PVN whose counter-regulatory influence upon the PVN CRH neuron results in the stress nonresponsive period.

Summary and Clinical Implications of Our Data in Lew/N and F344/N Rats:

Taken together, our data provide compelling evidence for a general principle underlying susceptibility to inflammatory and autoimmune disease: that the defective HPA axis response to inflammatory and immune mediators is a critical element in susceptibility to inflammatory/autoimmune disease. Thus the appropriate immune response genes allow the organism to recognize and react to antigenic or pro-inflammatory triggers with an appropriate immune/inflammatory response, while an intact HPA axis modulates the intensity of that immune/inflammatory response. The corresponding behavioral and HPA axis responses to

behavioral stressors of LEW/N rats suggest that both susceptibility to inflammatory disease and non-adaptive behavioral responses to stress may be related to the same underlying neuroendocrine response patterns. If the principle also applies to susceptibility to autoimmune/inflammatory and behavioral disorders in humans, it will provide new avenues for identifying individuals at risk for development of and associated affective disorders. In this regard, patients with illnesses such as rheumatoid arthritis have a very high incidence of a depressive-like syndrome that our group has shown to be associated with hypofunctioning of the CRH neuron. In addition, our model also makes feasible identification of the molecular and genetic defect(s) in the central nervous system which are associated with susceptibility to autoimmune/inflammatory diseases. For the first time it provides a controllable experimental system to examine the interactions between the immune and the central nervous systems at a molecular level and to elucidate at a molecular and genetic level the relationship between behavior and susceptibility to inflammatory disease.

Significance to the Biomedical Research Program of the Institute:

We have identified an animal model for susceptibility to inflammatory disease and differential behavioral responses to stress, related to a defect in regulation of hypothalamic-pituitary-adrenal responses to inflammatory and stress mediators. These data validate that a negative feedback loop exists between the immune and central nervous systems, in which immune/pro-inflammatory mediators signal the hypothalamic corticotropin releasing hormone (CRH) neuron to promote pituitary-adrenal activation and, hence, glucocorticoid mediated restraint of the immune response. Taken together, our data provide compelling evidence for a general principle underlying susceptibility to inflammatory and autoimmune disease: that the defective HPA axis response to inflammatory and immune mediators is a critical element in susceptibility to inflammatory/autoimmune disease. The ontogeny studies we have performed provide the basis for suggesting that these non-adaptive responses may occur very early in development in affected strains.

Our data also suggest that the CRH neuron in the rat plays a dual role in conferring an adaptive advantage during the stress of inflammation or an injury. Hence, cytokine-mediated CRH release seems to serve the functions of restraining the immune system from over-responding and promoting cautious avoidance to prevent further confrontation with potentially injurious encounters. The behavioral repertoire of the LEW/N rat is reminiscent of many features of the symptom complex of atypical depression. In this regard, atypical depression is a ubiquitous finding in patients with rheumatoid arthritis. Hence, we postulate that a deficient responsiveness of the CRH neuron may confer susceptibility to a combined syndrome of inflammatory arthritis and atypical depression, and that the behavioral and immunologic symptomatology associated with this syndrome may respond to treatment with neuropharmacologic agents.

If the principle also applies to susceptibility to autoimmune/inflammatory and behavioral disorders in humans, it will provide new avenues for identifying individuals at risk for development of inflammatory disease as well as potential new therapeutic approaches to autoimmune/inflammatory and associated affective disorders. This model also makes feasible identification of the molecular and genetic defect(s) in the central nervous system which are associated with susceptibility to autoimmune/inflammatory diseases. For the first time it provides a controllable experimental system to examine the interactions between the immune and the central nervous systems at a molecular level and to elucidate at a molecular level the relationship between behavior and susceptibility to inflammatory disease.

Future Directions:

The overall aim of future studies will focus on defining whether the differences in specific neurotransmitter/glucocorticoid feedback systems are the primary cause of LEW/N CRH hypo-responsiveness, or are secondary to an underlying common abnormality in CRH regulation. In this regard, an important aspect of the overall future aims is to define the nature of the genetic transmission of the phenotypes of HPA axis responsiveness, inflammatory susceptibility and behavioral responses to stress, and to determine whether any of the neurotransmitter receptor or type 1 and Type 2 glucocorticoid receptor differences we have observed, as well as any concordant genotype differences, are co-inherited with any or all of these phenotypes. The future directions of clinical studies are taken directly from findings in the animal studies, and the primary aim of these will be to define the role of HPA axis responsiveness in a variety of inflammatory diseases. The specific future directions of these studies based on findings of previous studies and pilot experiments are outlined below.

Biochemical, neuroendocrine and neuroanatomical extent of the central nervous system defect associated with susceptibility to arthritis and inflammatory disease in the LEW/N rat.

Future directions of our biochemical, neuroendocrine and neuroanatomical studies will build upon our initial observations of differences in both inhibitory neurotransmitter and neuroendocrine systems, such as GABA/benzodiazepine and glucocorticoid Type 1 and Type 2 receptor feedback, as well as stimulatory neurotransmitter systems, such as 5-HT1A. These systems will be further characterized, either by probing with oligonucleotide probes to determine the presence of RFLPs, (see 5-HT1A, below), or by evaluation of regulation of gene expression under a variety of conditions (see Type 1 and Type 2 glucocorticoid receptor), as appropriate.

Behavioral studies: Analysis of the association between neuroendocrine and behavioral differences in LEW/N and F344/N rats:

Behavioral differences in these two strains may result from the differences in their hypothalamic CRH responses, differences in their glucocorticoid responses, and/or differences in a variety of neurotransmitter/neuropeptide systems which are either influenced by or which may influence the HPA axis. Examples of the latter include the GABA/benzodiazepine receptor number which we have found differs in these strains, secondary to their differential corticosteroid levels. Analysis of the neuroendocrine mechanisms of behavioral differences in LEW/N and F344/N rats will therefore focus on evaluation of each of these neurotransmitter/neuropeptide and neuroendocrine systems on various aspects of behavior.

Since behavior is a complex phenomenon, different behavioral paradigms will be used to evaluate different components of behavioral responses to stress, and a variety of stressors will be used. Specific agonists and antagonists of specific neurotransmitter/neuropeptide and neuroendocrine systems will be used in these paradigms in order to define the role of each system in the behavioral pattern under study. Full dose response effects will be established in both strains, in order to determine whether there is a shift in the dose effect curve in one strain compared to the other. The role of CRH will be evaluated by further studies using i.c.v. administration of CRH to LEW/N and F344/N rats, and determination of their behavioral responses in the open field and in response to acoustic and tactile startle. The role of the differential glucocorticoid responses of the two strains in their differential acoustic and tactile startle responses strains will be determined by evaluation of the startle response in adrenalectomized and non-adrenalectomized animals, and in rats treated with a range of doses of dexamethasone. In all cases, the LEW/N and F344/N responses will also be compared to those of outbred HSD rats.

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Genetic control of LEW/N and F344/N behavioral responses to stress, and relationship to HPA axis responses and inflammatory disease susceptibility.

In order to determine the genetic control of LEW/N and F344/N behavioral responses to stress, and to define their relationship to HPA axis responses and inflammatory disease susceptibility, LEW/N x F344/N F1 and F2 hybrids will be bred, and behavioral responses in the acoustic and tactile startle paradigm, as well as the open field will be evaluated. These paradigms have been chosen since they are relatively non-invasive, can be performed within a barrier, and have been shown to distinguish the parental strains quantitatively, with great consistency and with minimal overlap. The behavioral phenotype of F1 and F2 offspring will be evaluated using these paradigms in stringently controlled conditions, at precisely the same day post-natal for all animals tested, in order to minimize the effects of environmental and developmental variables. The behavioral responses in these paradigms will then be compared to the HPA axis responses, as determined by both basal and stress induced plasma corticosterone, ACTH and CRH hypothalamic mRNA expression. F1 and F2 offspring, selected for their behavioral phenotype (closely matching the parental phenotype of either strain) will be evaluated for their arthritis response to SCW, as well as for their HPA axis responses to SCW, in order to determine whether the behavioral phenotype is transmitted genetically in conjunction with the inflammatory susceptibility phenotype and the HPA axis phenotype of the parental strains.

Clinical studies:

Based on the findings in the LEW/N and F344/N rat model, clinical studies have been directed at defining HPA axis responses in human inflammatory illness. Theoretically, perturbations of the immune system - CNS feedback loop at any level could render an individual susceptible to inflammatory disease. Furthermore, the concept suggests a category of disease states characterized both by inflammatory and emotional disturbances, which could result from genetic, infectious, toxic, or pharmacological disruptions of this bi-directional communication. In this regard, the pathophysiological perturbations in this bidirectional communication, and the category of disease states that result from these perturbations may confer susceptibility not only to inflammatory diseases such as rheumatoid arthritis and L-TRP EMS, but also on other diseases of the stress response, including major depression.

Studies to date have focused on patients with rheumatoid arthritis and L-tryptophan eosinophilia myalgia syndrome (see below). Initial parameters evaluated include baseline HPA axis function, including 24-hour Q 15-minute plasma ACTH and cortisol determinations, basal CSF CRH, and ACTH and cortisol responses to stimulation by graded doses of ACTH, CRH and AVP. Patients have undergone an extent of disease work-up to define activity of the immune system at the time of the HPA axis studies. Pending the outcome of our initial endocrinologic studies, further investigations may include the following: assessment of the petrosal sinus hormonal milieu; continuous sampling of lumbar CSF; assessment of the neuroendocrine responses to type I and type II GR agonists and /or antagonists; and the assessment of neuroendocrine responses to the administration of agents that activate the central component of the HPA axis (with and without pre-treatment with RU 486 to neutralize the impact of ambient glucocorticoid status).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02586 02 CNE
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Common Pathobiology of Eating, Obsessional, and Affective Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: Philip W. Gold, M.D., Chief, Clinical Neuroendocrinology Branch, NIMH Others: Dr. M.A. Altemus Staff Psychiatrist LCS, NIMH Dr. M.A. Demitrack Director, Eating Disorders Program, Univ. of Michigan Medical Center, Ann Arbor, MI Dr. J.R. Glowa Research Psychologist CNE, NIMH Dr. H.E. Gwirtsman Special Expert CNE, NIMH Dr. L.S. Brady Visiting Fellow CNE, NIMH Dr. M.A. Herkenham Research Psychologist SFN, NIMH Dr. M.A. Smith Medical Officer CNE, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Neuroendocrinology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 5.5	PROFESSIONAL: 4.0	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have developed a model suggesting that a confluence of the following four factors contribute to the susceptibility and natural history of <u>anorexia nervosa</u> and <u>bulimia nervosa</u>: (1) clinical and biochemical manifestations of obsessionalism; (2) a reduction in <u>metabolic rate</u> that interacts with obsessional features to produce a perseverative preoccupation with food intake and body image; (3) primary and/or secondary alterations in the neural mechanisms subserving hunger and satiety that reinforce pathological eating behaviors and; (4) an underlying depression that heightens the eating disordered patient's need to enhance low self-esteem by achieving an idealized body weight. In addition, we postulate that the abstinent bulimic experiences an unpleasant state of hypoaarousal not dissimilar from that seen in the various forms of atypical depression that is ameliorated by the bingeing and purging process. We have focused our studies on the neurobiology of obsessionalism in the eating disorders and classic <u>obsessive-compulsive disorder</u> OCD on three neurohormones, <u>arginine vasopressin</u> (AVP), <u>corticotropin releasing hormone</u> (CRH), and <u>somatostatin</u>. Our interest in AVP relates to the fact that administration of this neurohormone to experimental animals delays the extinction of behaviors acquired during aversive conditioning, an effect analogous to the obsessional patients' perseverative preoccupation with the potentially adverse consequences of relatively neutral stimuli or thoughts. Our data show that patients with classic OCD, bulimia nervosa, and anorexia nervosa all hypersecrete AVP into the CSF in association with profound disruptions of plasma AVP secretion. We also showed that among a wide range of antidepressants, only drugs preferentially capable of treating OCD (i.e. highly specific serotonin uptake inhibitors) significantly reduce AVP secretion into the CSF and diminish mRNA content and mRNA expression in the PVN of the rat hypothalamus. We also showed that these drugs also were unique in their capacity to reduce AVP release from rat <u>hypothalamic organ culture</u>. In addition to the hypersecretion of CSF AVP in classic OCD, we also found evidence of the hypersecretion of two other arousal producing neurohormones into the CSF, CRH and somatostatin. Our studies in patients with bulimia nervosa studied while actively bingeing and purging and following prolonged abstinence from these behaviors provide a model for the potential role of these behaviors in the economy of their illness. </p>		

Background and Summary of Research: Anorexia nervosa is a psychiatric syndrome characterized by a striking increase in physical activity and a marked diminution in food intake in the obsessive pursuit of thinness. This obsessive pursuit of thinness often dominates the patient's life to an extraordinary degree, so that life becomes literally organized around the rituals required to maintain a cachectic state. The mortality from this disorder either from the complications of cachexia or suicide is the highest of any psychiatric disorder. Our interest in this illness first arose from the observation that patients with anorexia nervosa invariably show hypothalamic-pituitary dysfunction. In the course of attempting to conceptualize the biological and psychological factors that interact to promote susceptibility to the development, maintenance, and treatment resistance of anorexia nervosa, we helped to clarify the mechanisms underlying the hypercortisolism, central dysregulation of water metabolism, and the hypersecretion of growth hormone in this disorder. Bulimia nervosa can be an equally debilitating disorder characterized by an uncontrollable pattern of recurrent binge eating and purging. Most patients with bulimia nervosa have strong appetites, cravings for food, and difficulty controlling eating after a meal. Like anorexia nervosa patients, bulimic patients are also pre-occupied with an obsessive pursuit of thinness, and their incapacity to refrain from binge-eating behavior has caused some to refer to them as failed anorexics. During periods of abstinence from bingeing and purging, patients with bulimia nervosa often complain of profound lethargy and fatigue. Bulimia nervosa is a common illness, and some have estimated that 25% of the college-age female population manifest some form of the disorder. Both patients with anorexia nervosa and bulimia nervosa have a greater than expected family history for major depression.

We have developed a model suggesting that a confluence of the following four factors contribute to the susceptibility and natural history of these illnesses: (1) clinical and biochemical manifestations of obsessionalism; (2) a reduction in metabolic rate that interacts with obsessional features to produce a perseverative preoccupation with food intake and body image; (3) primary and/or secondary alterations in the neural mechanisms subserving hunger and satiety that reinforce pathological eating behaviors and; (4) an underlying depression that heightens the eating disordered patient's need to enhance low self-esteem by achieving an idealized body weight. In addition, we postulate that the abstinent bulimic experiences an unpleasant state of hypoarousal not dissimilar from that seen in the various forms of atypical depression that is ameliorated by the bingeing and purging process.

Our data indicate that patients with anorexia nervosa and bulimia nervosa show obsessional symptoms in the range of severity seen in patients with classic obsessive compulsive disorder. These include behaviors such as ritual cleaning, trichotillomania, and repetitive checking. Moreover, their intense preoccupation with food intake and body image can be construed as an obsessional symptom. We have previously reported (*N. Eng. J. Med.*, 308:1117-1123, 1983) that patients with anorexia nervosa show a profound disruption in the osmoregulation of plasma arginine vasopressin associated with hypersecretion of this peptide into the cerebrospinal fluid. In the light of the fact that centrally-directed vasopressin in experimental animals delays the extinction of behaviors acquired during aversive conditioning, we postulated that the hypersecretion of vasopressin into the CNS could contribute to the obsessive preoccupation that patients with anorexia nervosa have with the potential adverse consequences of eating and weight gain. In the past year, we have shown that normal weight patients with bulimia nervosa and patients with classic obsessive compulsive disorder show qualitatively similar abnormalities in the osmoregulation of plasma arginine vasopressin and in the secretion of this peptide into the CSF. We also identified additional defects in the regulation of CRH and somatostatin secretion in patients with classic obsessive disorder that may be of further relevance to the symptom complex of this disorder.

Our data show that normal weight patients with both anorexia nervosa and bulimia nervosa show a reduction in metabolic rate that predisposes them to weight gain, and hence, to potentially pathologic mechanisms for maintaining thinness. In patients with bulimia nervosa studied during a phase of abstinence from bingeing and vomiting, this reduced metabolic rate is associated with a decrement in sympathetic function, augmented parasympathetic tone, and decreased thyroid function. During the phase of active bingeing and vomiting, there is a reversal of each of these defects that may reinforce this pathologic behavior. We postulate that a binge-purge reversible diathesis to weight gain superimposed upon clinical and biochemical manifestations of obsessionalism constitute a particularly toxic confluence that impels patients with the eating disorders into a style of life dominated by pathological eating behavior.

We have recently completed a series of studies exploring neural mechanisms underlying hunger and satiety in patients with the eating disorders. Our data show that patients with both bulimia nervosa and anorexia nervosa show defects in hunger and satiety that either confer susceptibility to their illnesses or complicate recovery. As an example, Dr. Thomas Geriocioti had discovered that patients with bulimia nervosa showed deficient CCK secretion and subjective satiety during the consumption of a normal sized meal that correlated strongly with a deficient sense of satiety after eating. However, during a binge-sized meal, we showed that CCK secretion and subjective satiety were normal. These data suggest that the pathological eating behavior in patients with bulimia nervosa, perhaps as a partial consequence of metabolic and obsessional factors, promoted secondary disturbances in appetite regulation that reinforce abnormal patterns of food intake.

One additional approach we have taken to the study of patients with eating disorders has been to compare and contrast pathophysiological mechanisms in these subjects and those with major affective disorders. As an example, in back-to-back original articles in *The New England Journal of Medicine*, we showed that the hypercortisolism in patients with anorexia nervosa (*N. Eng. J. Med.*, 314:1335-1342, 1986) and melancholic depression (*N. Eng. J. Med.*, 314:1314-1326-1135) had a similar etiology in the hypersecretion of CRH, and that this defect could contribute to many of their common clinical and biochemical manifestations. On the other hand, the depressive symptomatology in abstinent patients with bulimia nervosa that more closely resembles the syndrome of atypical depression may be alleviated by the repetitive bingeing and vomiting that raises sympathetic tone, augments thyroid function, and activates the hypothalamic-pituitary-adrenal axis.

Common Clinical and Biochemical Features in Patients with Anorexia Nervosa, Bulimia Nervosa, and Classic Obsessive Compulsive Disorder.

1. Overlap in Symptomatology Between Eating and Obsessional Disorders

In collaboration with Drs. Dennis Murphy and Terry Pigott, Dr. Margaret Altemus demonstrated that patients with bulimia nervosa and anorexia nervosa show obsessional symptoms in the range of severity seen in patients with classic obsessive-compulsive disorder. These include behaviors such as ritual cleaning, trichotillomania, and repetitive checking. Moreover, their intense preoccupation with food intake and body image can be construed as an obsessional symptom. Conversely, both male and female patients with classic obsessive compulsive disorder are obsessively preoccupied with a drive for body thinness and dissatisfaction with body image, as assessed by the Eating Disorders Inventory.

2. *A common defect in the regulation of plasma and cerebrospinal fluid arginine vasopressin (AVP) secretion in the eating and obsessional disorders*

Plasma and CSF AVP Secretion in Anorexia Nervosa:

We have previously reported that patients with anorexia nervosa show a profound disruption in the osmoregulation of plasma arginine vasopressin that had only been previously described in patients with hypothalamic neoplasms ablating the hypothalamic osmoreceptor while sparing vasopressin neurosecretory neurons. Hence, in response to the sustained intravenous infusion of hypertonic saline, the majority of underweight patients with anorexia nervosa fail to show the expected smooth rise in plasma vasopressin secretion, but rather demonstrate erratic secretion of vasopressin entirely dissociated from osmoreceptor control. A second, less common defect in underweight anorexics was a subnormal but osmotically-mediated release of vasopressin in response to osmotic stimulation, resembling the pattern classically seen in neurogenic diabetes insipidus. Both defects were associated with significant polyuria. We also showed that this defect in the osmoregulation of plasma arginine vasopressin secretion was closely linked to hypersecretion of arginine vasopressin into the cerebrospinal fluid. In the light of data showing that centrally-directed vasopressin in experimental animals delays the extinction of behaviors acquired during aversive conditioning, we suggested that the hypersecretion of arginine vasopressin into the CNS could contribute to the obsessive preoccupation patients with anorexia nervosa have with the potential adverse consequences of eating and weight gain. Neither the defects in plasma or cerebrospinal fluid vasopressin secretion resolved quickly after the resolution of weight gain, and in some instances had persisted for as long as a year despite maintenance of normal weight.

Plasma and CSF AVP Secretion in Bulimia Nervosa:

We have recently shown that normal weight patients with bulimia nervosa also show either a disruption of osmotically-mediated vasopressin secretion into plasma, or more commonly, a subnormal but osmotically-mediated response indicative of neurogenic diabetes insipidus. As with anorexia nervosa patients, both defects were associated with significant polyuria. Moreover, there was an associated significant increase in the CSF levels of vasopressin. We also reported that bulimic patients showed increased thirst that correlated positively with the levels of vasopressin in the CSF.

Plasma and CSF AVP Secretion in Classic Obsessive-Compulsive Disorder:

Dr. Margaret Altemus has advanced our most recent data showing that patients with classic obsessive-compulsive disorder also show abnormalities in the osmoregulation of plasma arginine vasopressin secretion that take one of two forms: in half, there was a disruption of osmotically-mediated vasopressin secretion into plasma; the remaining patients showed osmotically-mediated vasopressin secretion into plasma with an increase rather than a decrease in the sensitivity of the osmoreceptor. The abnormal osmoregulation of plasma arginine vasopressin in patients with classic obsessive-compulsive disorder was also associated with significant hypersecretion of this peptide into the cerebrospinal fluid.

Taken together, these data suggest that abnormalities in the regulation of vasopressin secretion into the plasma and cerebrospinal fluid are a consistent part of syndromes associated with significant obsessional symptomatology. These data also indicate that anorexia nervosa and bulimia nervosa share significant pathophysiological features with each other and with classic obsessive-compulsive disorder.

3. Abnormalities of oxytocin secretion in anorexia nervosa of possible relevance to the obsessionalism of this disorder

Oxytocin is a neuropeptide whose structure closely resembles that of vasopressin. Many studies indicate that the behavioral and endocrine effects of oxytocin are antithetical to those of vasopressin. Hence, the central administration of oxytocin enhances rather than delays the extinction of behaviors acquired during aversive conditioning, and oxytocin has been termed by some as an amnesic neuropeptide. Our data show a significant reduction in the levels of oxytocin in the cerebrospinal fluid of patients with anorexia nervosa who consistently restrict food intake. On the other hand, the levels of CSF oxytocin were normal in patients with anorexia nervosa and bulimia nervosa who binge and vomit as a means of controlling weight gain. The restrictor anorexics are thought to represent the most obsessional and controlled of the patients with the eating disorders, while bingeing and vomiting anorexics and bulimics have been referred to anecdotally as failed restrictor anorexics. In this regard, it is of potential interest that the most obsessional of the patients with an eating disorder preferentially shows an additional biological abnormality that could theoretically work together with the hypersecretion of centrally-directed arginine vasopressin to promote obsessional behavior.

4. Defects in the secretion of CSF AVP secretion in obsessive-compulsive disorder correlate with abnormalities in the secretion of CSF somatostatin and corticotropin releasing hormone-CSF AVP and Somatostatin in Patients with Obsessive Compulsive Disorder

Dr. Margaret Altemus has recently discovered that the CSF levels of somatostatin are significantly increased in patients with classic obsessive compulsive disorder. These data are of interest in the light of experimental studies showing that like vasopressin, somatostatin delays the extinction of behaviors acquired during aversive conditioning. In this regard, the levels of CSF vasopressin and CSF somatostatin are positively correlated with one another in controls and patients with obsessive compulsive disorder. Pre-clinical data show that the effects of centrally-administered somatostatin can be abolished by the co-administration of icv vasopressin antisera. Additional pre-clinical data indicate that the chronic administration of clomipramine is associated with a decrease in somatostatin content in several central loci, in contrast to the effects of other antidepressant medications.

Corticotropin Releasing Hormone in Obsessive-Compulsive Disorder

Dr. Altemus has also shown that the levels of CSF CRH are significantly elevated in patients with obsessive-compulsive disorder and has postulated that this abnormality is compatible with the profound hyperarousal that characterizes the obsessive compulsive patient's unbound anxiety when obsessive thoughts and compulsive rituals are not performed. The levels of both CSF somatostatin and CSF vasopressin were positively correlated with the levels of CSF CRH in patients with obsessive-compulsive disorder and in controls. These data are of interest in the light of findings that CRH promotes the release of somatostatin in the CNS, while vasopressin promotes the release of CRH. Moreover, both somatostatin and vasopressin also promote arousal, and in the context of CRH hypersecretion, may contribute to the particularly high level of anxiety that is the constant hallmark of severe obsessive-compulsive disorder.

We cannot definitively account for the fact that elevated CSF CRH levels in melancholia are associated with a concomitant decrease in the levels of CSF somatostatin and vasopressin rather than the increase in the levels of these CSF neuropeptides. In the light of the fact that glucocorticoids profoundly suppress the secretion of somatostatin and vasopressin into the CSF, we speculate that this may reflect the presence of pronounced hypercortisolism in melancholic depression, a feature not found in obsessive-compulsive disorder. We also cannot definitively account for the finding of eucortisolism in patients with OCD in the context of elevated CSF CRH

levels. Such a defect could reflect intrinsic defects in the pituitary and/or adrenocortical responsiveness to the stimulation from above, a defect that could enhance the arousal associated with OCD. Alternatively, if the increased secretion of somatostatin were primary in OCD, then such a defect could suppress CRH-induced ACTH release and interfere with the establishment of a centrally-mediated hypercortisolism.

5. An Animal Model of Food-Deprivation Induced Hyperactivity and Obsessionality:

An additional animal model of anorexia nervosa currently under study in our laboratory is that in the rat, in which food deprivation or restriction of the time of food presentation to a few hours per day results in a profound increase utilization of a running wheel, with locomotion leading to exhaustion, and at times, death. This model is of potential interest because along with food deprivation, compulsive exercise is the hallmark of anorexia nervosa. We are currently studying patterns of gene expression and other neurobiological parameters in this model, as well as exploring a means for its pharmacological reversal. This model could also be construed as a model of obsessionality as well. In this regard, Dr. Altemus has shown that a four-week trial of Prozac abolished food-deprivation-induced hyperactivity.

Clinical Evidence for Intrinsic Disturbances in Metabolic Rate (and Arousal) in Patients with Bulimia Nervosa that are Modifiable by Bingeing and Vomiting *Factors contributing to the low metabolic rate in abstinent, normal weight bulimics:*

Dr. Margaret Altemus has advanced data that patients with bulimia nervosa have a low metabolic rate that may predispose them to weight gain, and hence to pathological eating behaviors. The following four factors contributed to this lowered metabolic rate:

1. Decrease in the function of the sympathetic nervous system in bulimia nervosa:

Dr. Margaret Altemus has shown that abstinent normal weight bulimics show a decrease in sympathetic tone as assessed by estimation of basal norepinephrine spillover into arterial blood. Moreover, during the presentation of a meal, there was a significant blunting of the norepinephrine spillover rate. Dr. Altemus has also shown that the levels of arterial epinephrine (epinephrine is not reliably measurable in venous blood) rise during feeding in controls and the rise is also blunted in bulimia. She is currently analyzing metabolic rate responses to graded doses of IV epinephrine in a separate group of controls and in bulimics to see whether the differences in arterial epinephrine and norepinephrine spillover cause a blunting of the thermic effect of food or whether bulimics hyperrespond to epinephrine or norepinephrine because of increased receptor sensitivity.

2. Increase in the function of the parasympathetic nervous system in bulimia nervosa:

Dr. Altemus has also shown that normal weight abstinent bulimics show a significant increase in resting vagal tone, as estimated by time series analysis of heart rate variability. This finding could independently contribute to the reduction in metabolic rate seen in bulimia nervosa.

3. Decreased thyroid function in bulimia nervosa:

Dr. Altemus has shown that normal weight abstinent bulimic patients show a significant reduction in plasma free T4 concentrations in association with normal basal plasma TSH levels. At first glance, these data are suggestive of a central hypothyroidism, because in a primary hypothyroidism one would expect elevated basal levels of plasma TSH. On the other hand, assessment of the 24-hour pattern of plasma TSH secretion show a normal nocturnal TSH surge, in contrast to the usual pattern seen in central hypothyroidism. In the light of data that in some circumstances patients with central hypothyroidism secrete a normal quantity of immunoassayable TSH that is relatively biologically inactive, we cannot rule out a central hypothyroidism in these patients. These defects are associated with both a reduction in T3 and reverse T3; the latter is incompatible with a diagnosis of the euthyroid sick syndrome. Functionally, the magnitude of this

hypothyroidism is sufficient to contribute to the reduction in metabolic rate seen in bulimia nervosa; moreover in the light of our data that hypothyroidism is associated with a central CRH deficiency, this abnormality in thyroid function in abstinent bulimics may also contribute to their symptoms of atypical depression.

Normalization of sympathetic, parasympathetic, and thyroid function during active bingeing and purging:

Dr. Altemus studied patients with bulimia nervosa on our ward during a period of abstinence and during a sustained documented period of active bingeing and purging. During the latter phase, bulimic patients showed a significant increase in sympathetic function, a significant decrease in parasympathetic tone, and a significant increase in indices of thyroid function. The increase in norepinephrine spillover and arterial epinephrine secretion that occurred during this phase was associated with the persistence of an increase rather than a compensatory decrease in peripheral beta adrenergic tone. Moreover, during bingeing, patients had a 5-fold increase in the release of norepinephrine, apparently as a pre-absorptive response, because almost all ingested food from the binge was purged.

We cannot definitively account for the resolution of these defects during the phase of bingeing and vomiting. The activation of thyroid function and the increase in beta adrenergic tone in the context of increased catecholamine secretion could promote arousal in patients who complain of lethargy and fatigue during the abstinent state. Moreover, the consequent increase in metabolic rate without weight gain during the phase of bingeing and vomiting could reinforce pathological eating behaviors in bulimic patients.

Clinical Evidence for Primary and/or Secondary Alterations in Neural Mechanisms Subservicing Hunger and Satiety

1. Abnormal plasma CCK responses to a normal meal in bulimic patients:

Dr. Thomas Geraciotti had shown prior to his joining our group that patients with bulimia nervosa show a marked decrease in plasma cholecystokinin (CCK) responses during the administration of a test meal. CCK is a potent anorexogenic agent, so that a deficiency in CCK secretion during eating could contribute to the bingeing behavior of bulimia nervosa. The deficient CCK response in bulimia nervosa correlated with a deficient sense of satiety during food consumption.

2. Normalization of the plasma CCK secretion in bulimic patients during the presentation of a binge-sized meal:

In a study on our clinical research unit in which bulimia nervosa patients were studied longitudinally while abstinent and during a phase of active bingeing and vomiting, Dr. Margaret Altemus showed that the CCK responses to a test meal normalized during actual bingeing behavior. This suggests that the deficient CCK response to a normal meal in bulimia nervosa is secondary to the effects of prolonged bingeing and vomiting, and that CCK responses are normal in bulimics only during the presentation of very large portions of food. Such a secondary defect would serve to perpetuate the abnormal eating behavior in this disorder.

3. Peripherally-generated CCK activates the hypothalamic CRH neuron via peripheral vagal afferents: Clinical Implications:

Pre-clinical studies show that both CRH and CCK are potent anorexogenic agents, and that peripherally-administered CCK promotes both the release of ACTH and the induction of satiety. To explore the possible participation of CRH in the anorexogenic and pituitary-adrenal activating effects of peripherally and centrally-administered CCK, we first examined the effects of vagal deafferentiation and the administration of a peripherally-acting peptide CCK antagonist on IV CCK-induced ACTH release. Both vagal deafferentiation and blockade of CCK receptors on vagal

afferents abolished IV-CCK induced ACTH release. These studies indicated that peripherally administered CCK acted via vagal afferents to produce the release of a central secretagogue of ACTH. The fact that the administration of IV CRH antisera also blocked IV CCK-induced ACTH release indicated that this secretagogue was in fact CRH. We also showed that the icv administration of CCK, as well as incubation of hypothalamic organ culture with CCK, produced a dose-dependent release of CRH, while incubation of dispersed pituitary cells with CCK was not associated with ACTH release. Taken together, these data indicate that CRH seems to be involved in the pituitary-adrenal activation provoked both by the peripheral and central administration of CCK, and they raise the question that peripherally generated CCK may promote satiety, in part, by provoking the release of CRH. These data also suggest that CCK may be related to the mealtime-related pulse in ACTH secretion, and that deficient CCK release during feeding in bulimia nervosa may be associated not only with deficient satiety but also contribute to the hypoarousal that is part of the clinical picture of bulimia nervosa.

4. Studies on the Effects of the Repeated Injection of CRH into the Ventricular System of Non-Human Primates:

We have shown that the most profound effect of the icv administration of CRH to the rhesus monkey is the induction of anorexia. Moreover, we have shown that sensitization of the response to central CRH administration occurs with after as few as three consecutive daily doses, after which rhesus monkeys show persistent anorexia lasting for up to six weeks. This sensitization of the response to the repeated icv administration of CRH to the rhesus monkey has possible relevance not only to the natural history of affective illness, but also to the potential clinical relevance of the hypersecretion of CRH we have documented in patients with anorexia nervosa. Dr. Linda Brady showed that food deprivation in the rat decreases rather than increases the expression of CRH mRNA in the PVN, suggesting that one compensatory response to food deprivation is inhibition of an anorexogenic peptide. In this regard, the hypersecretion of CRH in anorexia nervosa may not simply reflect an artifact of chronic starvation.

5. CSF NPY levels in patients with anorexia nervosa

Patients with anorexia nervosa show increased levels of CSF neuropeptide Y, a neurohormone known to increase food consumption. Dr. Brady has shown that food deprivation in the rat causes an increase in the expression of NPY mRNA in the arcuate nucleus, suggesting that this finding in anorexia nervosa occurs as compensatory response to chronic food deprivation. This abnormality persisted after correction of the weight loss and was most prominent in patients who showed persistent amenorrhea, compatible with the antigonadotropic effects of this peptide in experimental animals.

6. A profound abnormality in the secretion of interleukin-1 into the CSF of patients with bulimia nervosa:

IL-1 is localized not only in the glial cells of the CNS, but in neurons of the PVN and elsewhere. In addition to its role as an inflammatory mediator, it is thought that central IL-1 has a variety of behavioral effects, including suppression of appetite. Recent unpublished data from our laboratory shows that CSF IL-1 levels are profoundly elevated (ten-fold) in patients with active bulimia nervosa. The pathophysiological significance of this finding is not clear, but it could reflect an effort to counter-regulate other defects promoting hyperphagia in bulimia nervosa. In this regard, it has been previously shown that the orexogenic neuropeptide PYY is highly elevated in the CSF of patients with bulimia nervosa.

7. Mapping IL-1 receptor antagonist mRNA in the CNS:

Many components of the IL-1 system are present in brain. We have demonstrated the presence of IL-1 mRNA in neurons of the PVN, while others have shown the presence of IL-1

receptors in disparate areas of brain. Recently a peptide has been cloned that is a specific antagonist to the IL-1 receptor, representing the first example of a pure endogenous peptide antagonist. Utilizing in situ hybridization, we have shown the presence of this IL-1 receptor antagonist mRNA in the PVN and other sites of potential relevance to the possible involvement of the IL-1 system in the eating disorders and other illnesses.

Comparison of Neuroendocrine Regulation in Patients with Major Depression and the Eating Disorders

1. CRH in the pathophysiology of hypercortisolism in anorexia nervosa and melancholic depression

Patients with both melancholic depression and anorexia nervosa can show hypercortisolism in the range of that seen in patients with Cushing's disease. Because hypersecretion of CRH could not only account for the confluence of hypercortisolism in patients with these disorders, but also for a variety of other common manifestations (e.g. anorexia, hypothalamic, decreased libido, anxiety), we sought to determine whether the hypersecretion of CRH was a common denominator in the hypercortisolism of these disorders.

We have previously reviewed our data regarding the potential role of CRH in the hypercortisolism of patients with major depression. We have advanced several similar lines of evidence indicating a role for CRH in the hypercortisolism of patients with anorexia nervosa. Hence, patients with anorexia nervosa show attenuated ACTH responses to synthetic CRH that correlate negatively with basal cortisol levels, normal basal levels of ACTH, and biochemical evidence of adrenal hyperresponsiveness to ACTH. Moreover, we have shown that patients with anorexia nervosa show frank elevations in CSF CRH levels that correlate positively with depression ratings and that fall towards normal with weight correction in association with resolution of hypercortisolism.

Our studies exploring the pituitary-adrenal responses to the antiglucocorticoid RU 38486 further support the idea that hypercortisolism in anorexia nervosa reflects hypersecretion of endogenous CRH. Hence, compared to controls, patients with anorexia nervosa show both an earlier onset of ACTH release during RU 38486 and a pituitary-adrenal response that is exaggerated. It has been previously demonstrated that RU 38486-induced ACTH release occurs only during the endogenous release of CRH. Moreover, the exaggerated pituitary-adrenal response to RU 38486 in patients with anorexia nervosa indicates that these patients do not show basal hypercortisolism as a consequence of glucocorticoid resistance, because they are able to show a greater than normal disinhibition of the hypothalamic-pituitary-adrenal axis in the context of glucocorticoid blockade.

2. Differential Regulation of Plasma and Cerebrospinal Fluid Vasopressin Secretion in Major Depression and Anorexia Nervosa

In contrast to the series of studies indicating that the pathophysiology of hypercortisolism in melancholic depression and anorexia nervosa reflect a common defect in the regulation of the CRH system, our data indicate that abnormalities in the secretion of arginine vasopressin differ in the two disorders. Hence, in contrast to evidence of disrupted osmoreceptor function associated with increased centrally directed arginine vasopressin secretion in patients with eating disorders, patients with major depression (non-psychotic) show osmotically mediated but subnormal vasopressin secretion during osmotic stimulation, secondary either to a decreased sensitivity of the osmoreceptor or a delay in the osmotic threshold for vasopressin secretion. This decrease in plasma vasopressin secretion is associated with a corollary decrease in vasopressin secretion into the CSF. An exception to the pattern of decreased vasopressin secretion into the plasma and CSF in depression is the markedly enhanced plasma and CSF vasopressin secretion which we have

consistently noted in psychotically depressed patients. These data are of potential interest in light of the fact that psychotic depressives can show the same kind of obsessive perseverative focus on issues relating to loss and personal deficiency as patients with eating disorders show on matters relating to food intake and body image.

3. *Differential Central Regulation of Growth Hormone Secretion in Major Depression and Anorexia nervosa*

Hypersecretion of growth hormone is a defect which is common to both major depression and anorexia nervosa. Our interest in this abnormality relates both to our wish to compare and contrast pathophysiological mechanisms in the affective and eating disorders and to the fact that GH-RH has behavioral as well as endocrine effects which consist of enhancement of food intake and sleep. In this regard, we postulate that GH-RH release during stress may represent an effort to counter-regulate the arousal producing and anorexogenic effects of CRH.

We have found that in patients with depression, GH responses to GH-RH were significantly attenuated in association with elevated levels of basal somatomedin C. In contrast, patients with anorexia nervosa showed exaggerated GH responses to GH-RH in association with a significant decrease in basal somatomedin C levels. We postulate that the blunted GH responses in depression reflect negative feedback inhibition of the pituitary somatotroph by elevated levels of somatomedin C. The elevation in somatomedin C strongly indicates that GH-RH is hypersecreted in depression to produce an increase in GH-mediated somatomedin C production. In contrast to depression, the GH response to exogenous GH-RH is disinhibited in anorexia nervosa as a consequence of the deficient levels and/or actions of somatomedin C. Such a deficiency in levels is likely a consequence of both cortisol-mediated suppression of GH-RH and somatomedin C.

In contrast to patients with major depression, CSF somatostatin levels in hypercortisolemic underweight patients with anorexia nervosa are similar to those seen in normal controls. Although we cannot definitively account for this finding, the significantly higher centrally directed CRH in anorexia nervosa compared to major depression could contribute to normalization of somatostatin secretion in anorexia nervosa. In this regard, several lines of data show that CRH stimulates the *in vivo* and *in vitro* secretion of somatostatin.

Significance to the Research Program of the Institute:

During the past year, we have developed new metaphors for conceptualizing the biological and psychological factors that interact to promote susceptibility to the development, maintenance, and treatment-resistance of patients with eating disorders. This work represents a major departure from that being done at other centers, and is supported by parallel studies in our basic laboratory, including development of novel animal models of eating disordered behaviors associated with compulsive exercise.

Our data show that both patients with anorexia nervosa and bulimia nervosa show an intrinsic reduction in metabolic rate that predisposes them to weight gain, and hence, to potentially pathologic mechanisms for maintaining thinness. In patients with bulimia nervosa, studied during a phase of abstinence from bingeing and vomiting, this reduced metabolic rate is associated with a decrement in sympathetic function, augmented parasympathetic tone, and decreased thyroid function. During the phase of active bingeing and vomiting, metabolic rate increases significantly in association with an augmentation in sympathetic tone, a decrease in parasympathetic tone, and an increase in thyroid function. We propose that this bingeing and vomiting-induced increase in metabolic rate reinforces this pathologic behavior and allows bulimic patients to correct an intrinsic metabolic defect without the liability of weight gain. The pre-absorptive mechanisms that promote

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01090-15 CNE						
PERIOD COVERED October 1, 1991 to September 30, 1992								
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Central Nervous System Functional Anatomy								
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> PI: Miles Herkenham Linda S. Brady </td> <td style="width: 33%; vertical-align: top;"> Research Psychologist Senior Staff Fellow </td> <td style="width: 33%; vertical-align: top;"> CNE, NIMH CNE, NIMH </td> </tr> </table> <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> Others: Allison B. Lynn Angelica Oviedo Ross A. Baker </td> <td style="width: 33%; vertical-align: top;"> Biologist HHMI Scholar IRTA Fellow </td> <td style="width: 33%; vertical-align: top;"> CNE, NIMH CNE, NIMH CNE, NIMH </td> </tr> </table>			PI: Miles Herkenham Linda S. Brady	Research Psychologist Senior Staff Fellow	CNE, NIMH CNE, NIMH	Others: Allison B. Lynn Angelica Oviedo Ross A. Baker	Biologist HHMI Scholar IRTA Fellow	CNE, NIMH CNE, NIMH CNE, NIMH
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SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;"> This Section combines molecular and neuroanatomical methods to reveal dynamic CNS events that relate to issues of mental health and drug abuse. Mapping <u>drug and neuro-transmitter receptors</u> using <u>autoradiography</u> has provided important basic information about brain organization and function. The <u>cannabinoid receptor</u> has been localized to specific neuronal elements in brain, and we examined its relationship to the <u>dopamine</u> system and to "reward" pathways. <u>In situ hybridization histochemistry</u> is used to examine the regulation of <u>gene expression of neuropeptides, monoamine transporters and synthe-sizing enzymes, and adrenal steroid receptors</u>. Studies are designed to elucidate brain mechanisms involved in <u>stress</u> and chronic <u>antidepressant</u> drug treatment, with a focus on the role of <u>corticotropin releasing hormone</u> in the pathophysiology of affective disorders. Other studies examine CNS adaptive changes caused by drugs of abuse, such as <u>marijuana</u> and <u>cocaine</u> </p>								

Objectives:

The localization of receptors for drugs, neurotransmitters, and other neuroactive substances is mapped in brain sections by the technique of in vitro receptor binding and quantitative autoradiography. One objective is to localize previously uncharacterized receptors and their subtypes in order to gain insights into their normal function. Another objective is to perform physiological and pharmacological manipulations in rats to demonstrate and localize receptor regulation or other changes underlying adaptive responses.

The localization and quantification of gene expression in brain is performed by the technique of in situ hybridization histochemistry. Expression of mRNAs for neuropeptides, synthesizing enzymes, and adrenal steroid receptors in nerve cells is examined. We perform pharmacological and physiological manipulations that affect the hypothalamic-pituitary-adrenal and noradrenergic systems. We have chosen these systems for study because of the well-characterized roles of 1) corticotropin releasing hormone (CRH), other hypothalamic neuropeptides, and receptors for adrenal steroids that negatively feedback onto brain, 2) the locus coeruleus noradrenergic system, thought to play a central role in coding stressful or novel features of stimuli.

Methods Employed:

We previously developed an in vitro autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue sections (the details of this technique are described in Project # Z01 MH 01090-09 LNP).

Dynamic activities in specified neurotransmitter systems are probed by the technique of quantitative in situ hybridization histochemistry 35S-labeled synthetic complementary oligonucleotide or ribonucleotide probes for mRNAs of interest (the details of this technique are described in Project # Z01 MH 01090-14 CNE).

Radioimmunoassay of plasma is used to quantify levels hormones secreted by the pituitary. Plasma is collected from trunk blood.

Lesions or placement of cannulae into the brain are surgically performed using stereotaxic coordinates and aseptic techniques in anesthetized animals. Facilities for precise behavioral control and measurement have been set up to permit study of animal models of stress.

Major Findings:Receptor Binding Studies

Mapping receptor localizations using in vitro binding and autoradiography techniques continues to yield important basic information about brain organization and function. In collaboration with Dr. Richard Rothman (NIDA), multiple kappa opiate receptor subtypes were characterized and mapped in rat and human brain. U69,593 and bremazocine were used as radioligands under subtype-selective conditions. Quantitative examination of bremazocine binding resolved two k_2 binding sites in both rat and human brain. The ligand-selectivity patterns of k_2 sites differ widely in guinea pig, rat, and human brain. These findings suggest that there may be considerable variation in the ligand recognition site of kappa opiate receptor subtypes among mammalian species.

Receptor autoradiography has been to localize the distribution of cannabinoid receptors in brain. We characterized and validated the binding of [3H]CP 55,940, a synthetic potent cannabinoid, in slide-mounted brain sections and described assay conditions to autoradiographically visualize the CNS distribution of cannabinoid receptors in a number of mammals, including humans. In order to determine the neuronal localization of these receptors, neurochemical lesions of key striatal afferent and efferent systems were made. Striatal neurons and efferent projections were selectively destroyed by unilateral infusion of ibotenic acid into the caudate-putamen. The nigrostriatal dopamine pathway was destroyed in another set of animals by infusion of 6-hydroxydopamine into the medial forebrain bundle. The data showed that cannabinoid receptors in the basal ganglia are neuronally located on striatal projection neurons, including their axons and terminals. Cannabinoid receptors are not localized on dopaminergic nigrostriatal cell bodies or terminals. Thus, the effects of cannabinoids on dopamine circuits thought to be common mediators of reward are indirect and different from those of drugs such as cocaine and morphine which directly affect extracellular dopamine levels and produce craving and powerful drug-seeking behavior.

The axonal localization of cannabinoid receptors was found in another structure containing dense receptors—the cerebellum. Using strains of mutant mice showing selective absence of either cerebellar Purkinje cells or granule cells, the patterns of cannabinoid receptor labeling in the homozygotes indicated that the predominant location of cerebellar cannabinoid receptors is the granule cell. Given the selective localization of receptors to the molecular layer, it was concluded that these receptors reside on axons and terminals of granule cell projections into the molecular layer.

Drug Abuse Studies

The effects of acute and chronic administration of drugs of abuse are studied in rats, monkeys, and humans. The effects of acute and chronic cannabinoid administration were described above. Studies of the behavioral effects of cocaine in squirrel monkeys are described below. In addition, we are interested in the effects of cocaine on transmitter, receptor, and transporter levels in relevant brain systems in rats. One study examined the possibility that a single

injection of a psychomotor stimulant drug such as cocaine, amphetamine, or GBR 12909 could alter the levels of striatal neuropeptide mRNA expression both acutely and over a prolonged period of time. Three conditions were examined: (1) an acute group, which provided information about neurochemical alterations immediately following first-time exposure to the stimulants; (2) a delayed group, which provided information about the prolonged (14 days) consequences of a single injection of the stimulant; and (3) a challenge group, which provided information about long-term sensitization or desensitization caused by the single drug injection as revealed by a second challenge injection 14 days later. The injections resulted in acute elevations in mRNA levels of dynorphin, enkephalin, and substance P, and these levels returned to or below baseline in the delayed group. A second challenge administration of the stimulants re-elevated the biosynthetic activity of enkephalin and substance P only. All three of the psychomotor stimulants caused greater effects on mRNA expression in the dorsolateral (sensorimotor/ associative) striatum than in the nucleus accumbens. This finding was similar to that seen in the striatum of human cocaine addicts (see below).

Molecular Neuroendocrinology Studies

We are continuing to focus on CRH and its role in the production of a coordinated series of centrally-mediated events collectively termed the stress response. Plasma is collected for radioimmunoassay of corticosterone and ACTH levels as indices of pituitary-adrenal activation. Brains are removed fresh and frozen for cryostat sectioning at several brain levels, notably the hypothalamus at the levels of the paraventricular and arcuate nuclei, the hippocampus, the pituitary, and the locus coeruleus. Currently we are localizing and quantifying mRNA expression of numerous hypothalamic peptides, tyrosine hydroxylase (TH), glucocorticoid and mineralocorticoid receptors, enzymes associated with transmitter synthesis or second messenger utilization, and transporters associated with transmitter uptake.

In one set of experiments (done in collaboration with Dr. Richard Kvetnansky, NINDS), immobilization was used as a potent stressor. The effects of acute and repeated exposure to immobilization stress were examined on mRNA expression of CRH in the hypothalamus and TH in the locus coeruleus-norepinephrine system, which are the principal effectors of the stress response. Acute immobilization stress increased mRNA levels of CRH in the paraventricular nucleus of the hypothalamus and pro-opiomelanocortin (POMC) in the anterior pituitary, and increased plasma ACTH and corticosterone, consistent with a rapid activation of the hypothalamic-pituitary-adrenal (HPA) axis. The changes in the HPA axis were accompanied by an acute stress-induced increase in mRNA levels of mineralocorticoid receptor in the hippocampus, mineralocorticoid receptor and glucocorticoid receptor in the hypothalamic paraventricular nucleus, and glucocorticoid receptor in the anterior pituitary. Acute immobilization stress also increased TH mRNA levels in the locus coeruleus, consistent with elevations in plasma catecholamines. Repeated immobilization stress produced an even greater increase in mRNA expression of CRH in the paraventricular nucleus and TH in the locus coeruleus. These changes were associated with an increase in mRNA levels of mineralocorticoid receptor in the paraventricular nucleus and an increase in plasma ACTH and corticosterone. Thus, mRNA expression of glucocorticoid and mineralocorticoid receptors

was differentially regulated in brain by acute and repeated immobilization stress. The capability of CRH-containing neurons in the hypothalamus and TH-containing neurons in the locus coeruleus to respond to repeated stimulation is suggestive of altered CNS feedback mechanisms under repeated stress conditions.

It is of interest that the CNS systems which are activated during repeated stress, namely the locus coeruleus-norepinephrine system and HPA axis, are dysregulated in melancholic depression. Acute and short-term (1–3 weeks) administration of antidepressant drugs alters the functional activity of nearly all neurotransmitter systems in brain, but none of these changes correlates with the 3–5 week time period required to achieve therapeutic efficacy in depressed patients. Identification of neuroanatomical systems that are altered only after long-term treatment with antidepressant drugs could potentially reveal the mechanism of action responsible for the delayed therapeutic efficacy of antidepressant drugs. Previously, we found that long-term (8 weeks) administration of imipramine, the prototypic tricyclic antidepressant, decreased mRNA levels of TH in the locus coeruleus, CRH in the paraventricular nucleus of the hypothalamus, and POMC in the anterior pituitary. These changes were associated with an increase in mRNA levels of the hippocampal mineralocorticoid receptor, which is thought to play a role in regulating low levels of steroids on the HPA axis. With the exception of a small change in TH mRNA expression in the locus coeruleus after 2 weeks of drug, none of these changes was evident as a consequence of short-term administration of imipramine. The time-dependent decrease in mRNA expression of CRH in the hypothalamus and the decrease in mRNA levels in TH in the locus coeruleus may be relevant to the therapeutic efficacy of tricyclic antidepressants because patients with major melancholic depression show an activation of brain CRH and locus coeruleus-norepinephrine systems that resolve with successful administration of tricyclic antidepressants over a prolonged period of time.

To test the generality of the findings with imipramine, antidepressant drugs with differing pharmacologic activities were examined on mRNA expression in brain. The activating antidepressants fluoxetine, idazoxan, and phenelzine were selected because in contrast to imipramine these drugs tend to be preferentially effective in the treatment of atypical depression, which is associated with signs of pathologic hypoarousal such as lethargy, fatigue, hypersomnia, and hyperphagia. The three drugs decreased CRH mRNA levels in the paraventricular nucleus of the hypothalamus, and the decreases occurred after long-term administration (8 weeks) but not after short-term administration (2 weeks). In contrast to imipramine, the activating antidepressants increased TH mRNA levels in the locus coeruleus after short-term and long-term administration. The preferential efficacy of activating antidepressants in atypical depression may reflect a capacity to increase TH mRNA expression in the locus coeruleus. These findings are in accordance with clinical observations that an underactive noradrenergic system could contribute to the lethargy and hypersomnia associated with atypical depression. The time-dependent decrease in CRH mRNA levels in the paraventricular nucleus induced by fluoxetine, idazoxan, and phenelzine extends this finding to different classes of antidepressant drugs, namely serotonin reuptake inhibitors, α_2 -adrenergic antagonists, and monoamine oxidase inhibitors. The reduction in CRH mRNA expression in

the hypothalamus may be one element relevant to the therapeutic efficacy of antidepressant agents in the treatment of various forms of major depression.

The HPA axis plays an important role in adaptation to stressful stimuli and in the maintenance of homeostasis. Aging is associated with altered pituitary-adrenal function and a decreased capacity to maintain homeostasis in response to stress. In collaboration with Dr. Giovanni Cizza (Developmental Endocrinology Branch, NICHD), we examined the functional activity of each component of the HPA axis in unstressed, aged Fischer 344/N rats (18–24 months of age). Aging was associated with progressive decreases in basal plasma ACTH and corticosterone levels. In the hypothalamus, there was a progressive age-related reduction in stimulated CRH release *in vitro*, CRH content, and CRH mRNA expression in the paraventricular nucleus. In the pituitary, there was a progressive age-related decrease in POMC mRNA expression in the anterior lobe associated with an increase in ACTH content. An age-related decrease in mineralocorticoid receptor mRNA levels was found in the hippocampal CA fields and dentate gyrus. Glucocorticoid receptor mRNA levels were relatively unchanged in the hippocampal subfields. These findings suggest that aging is associated with a progressive decrease in HPA axis function that is at least partially due to a hypothalamic CRH deficiency.

The hippocampus appears to be an important modulator of the negative feedback effects of glucocorticoids on the HPA axis. Alterations in hypothalamic CRH mRNA expression produced by stress and chronic antidepressant treatment appear to be associated with changes in mRNA expression of hippocampal corticosteroid receptors. However, there is no direct anatomical connection between the hippocampus and hypothalamic paraventricular nucleus, and little is known about the mechanism whereby hippocampal corticosteroid feedback affects hypothalamic CRH function. We have begun to examine the anatomy of the pathways mediating glucocorticoid feedback of the hippocampus on CRH mRNA expression in the hypothalamus. Surgical removal of the entire hippocampus has previously been shown by others to increase the expression of CRH mRNA in the paraventricular nucleus of the hypothalamus. It is not known, however, if hippocampal subfields CA1-4 or the dentate gyrus differentially alter mRNA expression of hypothalamic CRH. We therefore examined the effects of selective destruction of dentate gyrus granule cells, which send excitatory glutamatergic inputs to the CA subfields, on CRH expression in the paraventricular nucleus. To determine the possible involvement of steroid receptors in the regulation of CRH mRNA expression, we examined the effects of intrahippocampal colchicine on mRNA expression of mineralocorticoid and glucocorticoid receptors in hippocampal CA subfields. Colchicine produced a marked reduction in CRH mRNA levels in the paraventricular nucleus and a simultaneous decrease in mRNA expression of the mineralocorticoid receptor in the CA subfields. Glucocorticoid receptor mRNA levels were relatively unchanged in the CA subfields of colchicine-treated animals. These findings suggest that the dentate gyrus is important for maintenance of steroid hormone receptor mRNA levels in the hippocampus and CRH mRNA expression in the hypothalamic paraventricular nucleus.

Behavioral Studies

A number of behavioral studies were carried out by the Biopsychology Unit of the CNE. Dr. John Glowa headed that Unit, and last September he moved to the Laboratory of Medicinal Chemistry, NIDDK. A number of papers have been published in this last year, and their content is summarized below.

Dr. Glowa has reviewed the effects of CRH on food intake compared with its effects on performances maintained by food presentation, and contrasted with its effects on performances maintained by other events. Data assessing the effects of CRH administration on central neurotransmitter levels were compared with levels seen in clinical populations. The effect of CRH on food intake seen in animals is consistent with a putative role for CRH in clinical syndromes where appetite suppression is apparent. Since some of the effects of CRH on food intake are subject to pharmacological intervention, strategies directed at peptidergic mechanisms of psychiatric disorders should be explored.

Dr. Glowa designed a large animal intracerebral drug administration device to allow the delivery of drugs or other agents to discrete loci within the brains of monkeys while maintaining sterile conditions. It is an improvement over existing devices because it 1) maintains an absolute minimal dead space within the system, 2) is smaller in diameter (by approximately 80%) than existing shunt catheters, minimizing tissue damage during placement, 3) is easily secured and requires minimal clearance over the cranium, and 4) maintains a sterile seal between the brain and periphery. Preliminary studies indicate the device is well accepted and fully functional for periods up to a year. The device is intended for permanent implantation.

Different rat strains that exhibit large differences in HPA activity have been used to determine the role of the neuroendocrine system in susceptibility to autoimmune disease. Challenge with inflammatory stimuli, stressors, or specific drugs render the Lewis (LEW/N) strain of rats susceptible to autoimmune disease while its histocompatible control strain, the Fischer (F344/N) rat, is resistant. To characterize potential behavioral correlates of these differences, the amplitudes of the acoustic (ASR) and tactile (TSR) startle responses and the corticosterone response to acoustic startle stimuli were compared between LEW/N and F344/N rats, as well as outbred Harlan Sprague-Dawley rats. Startle stimuli elicited larger ASR and TSR in LEW/N rats than in F344/N rats, with Sprague-Dawley rats exhibiting an intermediate response. The ASR habituated at a similar rate in LEW/N and F344 rats, while the ASR did not habituate in Sprague-Dawley rats. After handling and placement in the startle chambers, the three strains did not differ in control levels of corticosterone. In contrast, exposure to acoustic startle stimuli increased corticosterone 5-fold in F344/N rats and 2-fold in Sprague-Dawley rats, but had no effect on corticosterone in LEW/N rats. These findings suggested an inverse relationship between the amplitude of the ASR and hypothalamic-pituitary-adrenal activation across strains. This relationship was further supported by a high negative correlation between corticosterone level and ASR amplitude within the F344/N group. Both LEW/N and F344/N strains were a) assessed for differences in behavioral and corticosterone responses to exposure to an open field, b) prepared with ventricular cannulae, and assessed

again in the open field after intraventricular infusions of saline or 3 μ g of CRH. Significant baseline differences in open field response and in the effects of CRH on rearing, grooming, and activity were found between these strains. These differences suggest that differences in endogenous CRH may form the basis for the differential susceptibility of these strains to autoimmune disease. Such differences may serve as an animal model for genetic determinants of relationships between CNS function and the immune system.

Squirrel monkeys were trained to respond under second-order schedules of food presentation and then exposed to either a self-administration (SA) or to a conditioned taste aversion (CTA) procedure. Initial exposure to stimuli associated with post-session administration of 0.3 mg/kg cocaine either maintained (SA) or suppressed (CTA) responding, respectively. The monkeys were then exposed to the alternate procedure. Initial exposure to CTA, decreased cocaine SA responding compared to rates seen with initial SA exposure. In contrast, with initial exposure to SA, the CTA procedure failed to suppress responding. Thus, prior exposure to either reinforcing or suppressant effects of cocaine altered the subsequent behavioral effects of that drug, suggesting a unique role of behavioral history in the abuse potential of cocaine.

Diethyl ether has anesthetic, neuroendocrine-stimulating, and abuse-potential properties, yet little is known of the concentrations over which these apparently diverse behavioral effects occur. Adult male NIH mice were exposed to a range of concentrations of ether (1000-30000 ppm) in order to characterize its effects on operant behavior and neuroendocrine activity. When responding was maintained under FI-60 sec schedules of milk presentation, 5- or 30-min exposures to less than 3000 ppm ether were without behavioral effect, 10000 ppm produced up to 300% increases in responding, and higher concentrations abolished responding. Exposure to a similar range of concentrations elevated ACTH and corticosterone levels in a dose- and time-dependent manner. Short (5 min) exposures elevated baseline levels of ACTH (18.2 pg/ml) to 310.5 pg/ml (~1700% of control) at 10000 ppm, whereas corticosterone was relatively unaffected. With 30 min of exposure to 10000 ppm ether, corticosterone increased maximally from 78.44 ng/ml to 559 ng/ml (~700% of control) and ACTH was increased to a lesser extent. The imidazobenzodiazepine, Ro 15-4513 decreased FI responding at doses greater than 3 mg/kg and attenuated the rate-increasing effects of diethyl ether at 1 mg/kg.

Significance to Biomedical Research and to the Program of the Institute:

Lack of association of cannabinoid receptors with dopamine neurons indicates that cannabinoids do not directly affect dopamine release associated with reward and drug seeking behavior. The localization of cannabinoid receptors to axons and nerve terminals in motor areas suggests therapeutic applications for movement disorders. Cannabinoids have been shown to be beneficial for some forms of dystonia, tremor, and spasticity. Further work may show the basis for the reported usefulness in controlling nausea and stimulating appetite in patients receiving chemotherapy for cancer or AIDS. Finally, the development of a cannabinoid antagonist could lead to additional therapeutic applications. The section binding assay can be used to screen the potencies of novel drugs and serve to identify cannabinoid receptor subtypes, which could lead to renewed interest in developing cannabinoid drugs without unwanted side effects. The assay is currently being used to show the nature and extent of receptor down-regulation associated with tolerance to cannabinoids.

The endocrinology focus is a greatly expanded research effort, with numerous approaches ongoing simultaneously. This effort represents a major bridge between the basic research of the SFN and the clinical studies of the CNE. Several studies address the common interests directly, such as our work on animal models of stress and the consequences of antidepressant drug treatment. These studies are aimed at a better understanding of the roles that particular neuropeptides and monoamines play in the disorders created by stressful conditions.

Proposed Course of the Project:

The development of a reliable and sensitive section-binding assay for cannabinoid receptors has allowed us to extend our work in this rapidly growing field. We plan to elucidate further the close association of cannabinoid receptors with the adenylate cyclase second messenger system, using the binding assay to reveal the effects of preincubations and incubations with ions, guanine nucleotides, and other allosteric effectors. We are examining the effects of chronic administration of Δ^9 -THC and synthetic cannabinoids in rats. Preliminary results indicate that psychoactive cannabinoids given daily for 2 weeks result in development of profound behavioral tolerance and a large decrement in cannabinoid receptor levels. We will pursue these findings further, and examine also the rate of recovery from the down-regulated state. Localization and quantification of cannabinoid receptor binding and gene expression in humans with Huntington's and Alzheimer's diseases is ongoing in two other collaborative studies (E. Richfield, Univ. of Rochester Neurology Unit; T. Westlake and A. Howlett, Dept. of Pharmacology, St. Louis Univ. School of Medicine).

Studies are in progress to examine the distribution of cannabinoid receptors in non-neuronal tissues. Previous work in other labs, using both the receptor binding and gene expression assays, has indicated that cannabinoid receptors are scarce to non-existent in peripheral tissues. Using section-binding and hybridization techniques on all of the major glands and tissues of

the respiratory, immune, circulatory, endocrine, exocrine, reproductive, excretory, and digestive systems, we hope to find selective binding and/or gene expression in subsets of tissues that will allow us to relate receptor localization data to a huge, complex, and contradictory literature on cannabinoid effects on these various systems. We are initially excited by our finding of specific binding in discrete components of the immune system.

Using the techniques of *in situ* hybridization histochemistry and *in vitro* ligand binding and autoradiography, we are examining molecular changes in the neostriatum of human subjects who died with a history of recent cocaine use. Selective alterations in mRNA levels of striatal neuropeptides were detected in cocaine subjects compared to controls, especially for the opiate peptides dynorphin and enkephalin. Reductions in the levels of μ opiate receptors and elevations in levels of κ opiate receptors were also found. Furthermore, dopamine uptake sites were reduced in the caudate and putamen of cocaine subjects. The greater magnitude of changes in the dorsolateral striatum (caudate and putamen) as opposed to the ventromedial striatum (nucleus accumbens) suggests that cocaine abuse preferentially disrupts the biosynthetic activity of striatal systems associated with sensorimotor and cognitive functioning. Based on the reductions of transmitter and receptor expression associated with euphoria, together with elevations in transmitter systems associated with dysphoria, we propose that the neurochemical profile of the human cocaine addict brain reflects one in a state of "craving." Additional collaborative work (with Y. Hurd, Clinical Brain Disorders Branch, NIMH) will focus on changes in the dopamine transporter to which cocaine binds to produce its euphoric effects. The availability of the ribonucleotide probe for the dopamine transporter gene will facilitate this investigation.

Studies are in progress to examine mRNA expression of hypothalamic neuropeptides in human brains of anorexics. One focus of future research will be to characterize neuropeptide and neurotransmitter mRNA expression in human brains from patients with melancholic depression and manic depressive illness. Another focus will be to further characterize the role of specific neuropeptide or neurotransmitter genes in the mechanism of action of antidepressant drugs. Molecular techniques will be used to make deletions in relevant genes such as the CRH gene. The herpes simplex virus can be used as a vector to introduce the altered gene into specific brain sites. Another strategy will be to use antisense technology to inactivate the gene of interest.

We have a long-term interest in examining the role of hypothalamic and extrahypothalamic brain structures that play a role in the determination of normal and abnormal activity in the HPA axis. Toward this end we are initiating experiments to 1) determine the best animal models of depression, 2) investigate the consequences of antidepressant drug and lithium administration and of electroconvulsive shock treatment, and 3) pinpoint the circuitry mediating the changes that occur when chronic stressful stimuli alter hypothalamic output levels by way of direct and indirect feedback mechanisms.

Because our hypotheses of antidepressant drug action are based on changes in mRNA expression in unstressed, eucortisolemic rats, studies are in progress to characterize the effects

of these agents in several animal models of depression. The models under investigation are a dominant/subordinate paradigm and olfactory bulbectomy. In the former model described by Dr. Caroline Blanchard (Univ. of Hawaii), groups of rats are placed within a naturalistic burrows system and immediately develop a social hierarchy. Within days subordinate animals show characteristic signs of depression and will die if left in the social environment. Collaborative studies with Dr. Blanchard are in progress. Another rat model of depression is the condition generated by bilateral olfactory bulbectomy, which produces neuroendocrine and behavioral alterations that are very similar to those seen in depressed patients. We plan to use this model to test the efficacy of antidepressant drugs and localize the responsive neurochemical systems in the brain.

The neuroanatomical systems involved in the mechanism of action of antidepressant agents will be further characterized in rats by examining the effects of therapeutic dopamine- and GABA-selective antidepressants, lithium which is effective in manic-depressive illness, and repeated daily electroconvulsive shock which is used primarily in depressions which are refractory to treatment with antidepressant drugs.

Several studies are underway which are aimed at identifying the relevant circuits involved in mediating glucocorticoid negative feedback, via the hippocampus, to the hypothalamic CRH neurons. The anatomy of the pathways mediating glucocorticoid feedback of the hippocampus on CRH in the hypothalamus will be studied by making selective lesions of hippocampal subfields. Studies are in progress to examine the effects of selective destruction of hippocampal CA3 neurons with kainic acid and destruction of CA1 neurons as a result of transient cerebral ischemia.

The highest levels of the mineralocorticoid receptor found in the brain are in the hippocampus and lateral septum, whereas the highest levels of the glucocorticoid receptor are in the arcuate nucleus of the mediobasal hypothalamus, paraventricular hypothalamic nucleus, and brain stem catecholaminergic and serotonergic cell groups. A recent study of the functional relationship of these brain regions to CRH neurons showed that when dexamethasone was implanted above the arcuate nucleus, lateral septum or dorsal hippocampus, it restored levels of ACTH to near normal in adrenalectomized rats. Clearly, several brain regions are responsible for corticosteroid feedback regulation of CRH neurons. All the brain regions thought to be involved in corticosteroid feedback project to the arcuate nucleus, which in turn projects to the paraventricular nucleus. The extensive connections of the arcuate, coupled with the rich diversity of neurotransmitters synthesized by arcuate neurons, make it an attractive candidate for a major role in integration of the return to homeostasis following environmental stress. In order to understand how the arcuate nucleus may mediate corticosteroid feedback, we plan to: 1) investigate the adrenal steroid sensitivity of peptide/ neurotransmitter mRNA levels in the arcuate nucleus of adrenalectomized rats, 2) map the topography of those adrenal steroid-sensitive neurons, and 3) identify projections of corticosteroid-sensitive, transmitter-specific neurons from the arcuate to the parvocellular paraventricular nucleus, site of CRH producing neurons. Knowledge of the neural and hormonal inputs by which the arcuate may integrate external stimuli has broad application to the study of the brain's ability to respond to stress.

Based on results of the experiments described above, future studies will be aimed at further characterizing the integrative capacity of arcuate neurons. The question of how they respond to antidepressant drugs is especially interesting. In particular, changes in mRNA levels in identified corticosteroid-sensitive arcuate-paraventricular projection neurons after antidepressant drug treatment will identify chemically defined pathways that may contribute to the altered CRH neuron activity seen in depressive illness. Overall, the work will provide a greater understanding of the neurotransmitters and neuropeptides involved in the brain's integrated response to stress and in the pathophysiology of depression. The identification of specific, neurochemically defined pathways that regulate the stress response will hopefully lead to development of more specific and effective treatments for depressive illness.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02587-02CNE

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Neuropeptide
Gene Expression by Viral and Physiologic Modulators

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Jay S. Joshi, Ph.D., Biologist, CNE, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroendocrinology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Project terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02618 01 CNE
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) L-Tryptophan Eosinophilia Myalgia Syndrome: Etiology and Pathogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Esther M. Sternberg, M.D., Chief, Unit on Neuroendocrine Immunology, Clinical Neuroendocrinology Branch, NIMH		
Others: Dr. P.W. Burnet Dr. J.B. Joshi Dr. S.T. Koutmos Dr. D. Michelson	Visiting Fellow Biologist Guest Researcher Guest Researcher	CNE, NIMH CNE, NIMH CNE, NIMH CNE, NIMH
(Continued on p. 2)		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Neuroendocrinology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 4.5	PROFESSIONAL: 3.5	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>L-tryptophan eosinophilia myalgia syndrome</u> occurred in epidemic proportions in association with contaminated L-tryptophan. We first described this syndrome and studied its biochemistry in humans in relation to L-5-hydroxytryptophan in 1980 and again in relation to L-tryptophan in 1990. We have established an <u>animal model</u> for this syndrome in Lewis (LEW/N) rats, and have determined that one of the <u>impurities, (EBT)</u>, causes <u>fascial thickening</u> and together with pure L-tryptophan, causes peripheral blood <u>mononuclear cell activation</u> in LEW/N rats. Both EBT and contaminated L-TRP were associated with significant <u>suppression of hypothalamic CRH mRNA</u> expression, compared to rats treated with pure L-TRP or vehicle control. Furthermore, pure L-tryptophan itself, as well as contaminated L-TRP and EBT, cause <u>pancreatic fibrosis</u> in LEW/N rats. Thus, pure tryptophan contributes to some of the features of the syndrome, but alone does not cause its characteristic features. The breakdown product of this impurity, a <u>tetrahydro-beta-carbolene</u>, exerts variable effects on neuronal survival, depending on the stage of maturation of the cells. It is <u>toxic to mature neurons</u>, and <u>prevents naturally occurring cell death in immature cultures</u>, in a dose-related, stereospecific manner, suggesting that it exerts these effects through a <u>receptor mediated mechanism</u>. The neurotoxicity is dependent on <u>IL-1</u>. Taken together, L-TRP EMS may be viewed as a toxic syndrome in which a neurotransmitter-related molecule may both induce inflammatory disease, and amplify inflammation indirectly through suppression of the HPA axis. </p>		

(continued from P. 1)

Principal Investigator:

Others:	Dr. B. Poltorak	Visiting Fellow	CNE, NIMH
	Mr. C.C. Smith	Chemist	CNE, NIMH
	Dr. E. Zelazowski	Professional Service	CNE, NIMH
		Contract	
	Dr. P. Zelazowski	Visiting Fellow	CNE, NIMH

Specific Aims:

- (1) To determine the etiology and pathogenesis of the L-tryptophan related eosinophilia myalgia syndrome (L-TRP EMS).
- (2) To define the extent and characteristics of disease induced in LEW/N rats by the impure, implicated L-TRP and its specific synthetic impurities, and by non-implicated L-TRP.
- (3) To determine the role of the HPA axis in susceptibility to and course of L-TRP EMS.
- (4) To define the effect of synthetic L-TRP impurities on the HPA axis and CNS.
- (5) To define the molecular mechanism of induction of autoimmune/inflammatory disease by the neurotransmitter-like molecules implicated in the etiology of L-TRP EMS.
- (6) To identify specific antagonists to the synthetic impurities implicated in causing L-TRP EMS which could be used to treat L-TRP EMS and related syndromes.
- (7) To identify the endogenous receptor to which the specific impurity binds, and to clone this receptor.

Methods employed: A variety of techniques have been developed and/or are applied on the clinical research unit for the clinical study of patients with TRP EMS and other inflammatory diseases, and controls, including methods for the assessment of circadian pattern of neurohormone, neurotransmitter and neuropeptide release into cerebrospinal fluid, assessment of the cortisol and ACTH production rates, and many paradigms for the assessment of the functional integrity of each component of the hypothalamic pituitary-adrenal, gonadal, and thyroid axes. In the laboratory, we have raised antisera to a variety of neuropeptides including ovine and rat/human CRH, ACTH and various fragments, beta-endorphin, atrial natriuretic factor, arginine vasopressin, oxytocin, dynorphin, tumor necrosis factor, interleukin 1, interleukin 2, interleukin 6, neuropeptide Y, neuropeptide YY, cholecystokinin, and met-enkephalin. These antisera are used for radioimmunoassay, immunohistochemistry, and immunoneutralization studies. Affinity purification of antibody and immunohistochemistry procedures for these peptides have also been established. We also utilize gel and HPLC chromatographic methods for purification and identification of ovine and rat/human CRH, and for POMC fragments. Specific peptide antagonists for CRH, arginine vasopressin receptor subtypes, oxytocin, and cholecystokinin are also utilized. Additional methodologies include high resolution autoradiography, an ACTH bioassay in which rat adrenal corticosterone is examined as an endpoint, dispersed anterior pituitary cell cultures for examination of ACTH activity or various extrahypothalamic substances with ACTH bioactivity but not immunoreactivity, and a hypothalamic organ culture system for the assessment of factors regulating the acute release of CRH, TRH, and arginine vasopressin. We also employ an intravenously cannulated rat preparation with chronic maintenance and a system for maintenance of chronic central venous catheters in both the rat and non-human primate. Chronic intraventricular cannulae are also maintained in operantly conditioned non-human primates for studies of the behavioral effects of centrally-administered neuropeptides. Molecular methodologies include *in situ* hybridization, Northern blotting, transient and stable transfections, and standard cloning and sequencing procedures applied to the study of the type I glucocorticoid receptor and enkephalin genes. Behavioral methodologies include open field testing, acoustic and tactile startle response, swim and restraint studies. Pathology techniques include standard light microscopy, as well as special immunohistochemistry staining methods, and standard differential counts as well as FACS analysis of peripheral blood.

Background and summary of work in progress:

In November 1989, an epidemic of severe myalgia and eosinophilia was reported to the CDC and the New Mexico State Health Department. The syndrome occurred in patients who had taken L-tryptophan (L-TRP) which was readily available over-the-counter, and prescribed for problems including sleep and depression. Ultimately over 1500 patients in the United States were found to be affected, and to date, 31 deaths have been attributed to the disease. Patients were

reported in all 50 states, but predominated in Western states, including California, Washington, Oregon and New Mexico and also clustered in New York, New Hampshire, Minnesota and South Carolina. The distribution of cases is thought to reflect the usage pattern of L-TRP containing products. The epidemic peaked in November 1989, and dramatically fell at the time of the FDA recall of L-TRP, in November 1989. The epidemiologic data strongly indicated that the syndrome was linked to ingestion of L-TRP manufactured by a single Japanese company, Showa Denko, K.K. (SD). The majority of persons who became ill took L-TRP which had been manufactured during a limited period of time preceding the epidemic in the fall of 1988 to summer of 1989. During this period, SD had simultaneously changed several steps in the manufacturing process of L-TRP, including introduction of a new strain of bacteria, and alteration in the filtration procedure used to purify the compound.

Chemical analyses indicated that the implicated L-TRP contained more than 30 compounds in addition to L-TRP, including compounds structurally related to L-TRP (indoles and beta carbolines), and compounds related to antibiotics (bacitracin). Although several of these compounds in the L-TRP preparation were associated with the development of EMS, one of these compounds, called Peak 97, or Peak E, was most highly statistically associated. Peak 97 was identified as a molecule composed of two tryptophans linked together, now called 1,1'-ethylidenbis[tryptophan] or EBT. In the acid conditions of the stomach, at pH 2, EBT decomposes to a racemic mixture of 1S, 3S tetrahydro β carboline 3 carboxylic acid (SbC) and 1R, 3S tetrahydro β carboline 3 carboxylic acid (RbC).

While the LEW/N - F344/N rat model provides a tool for understanding some of the mechanisms and consequences of genetic perturbations of the immune system - CNS counter-regulatory loop, the L-tryptophan eosinophilia myalgia syndrome (L-TRP EMS) provides a tool for defining the mechanisms and consequences of chemical or toxicological interruptions of this loop. In this regard, L-TRP EMS may represent a prototypic inflammatory/autoimmune disease initiated by exposure to a neurotransmitter-related compound. Thus, the second major focus of this lab has been directed at analysis of the pathogenesis of the L-tryptophan eosinophilia myalgia syndrome at a clinical, toxicological and molecular level. These studies also have important public health implications not only in terms of understanding the causes of this epidemic, related to ingestion of impure L-TRP, but also in preventing such future occurrences and in development of specific treatment of the current syndrome. In addition, our data suggesting that the L-TRP EMS could be triggered by the activation of an endogenous receptor (*vide infra*) may provide information regarding the pathogenesis of a variety of fibrosing illnesses and provide new means for their treatment.

Our specific studies in the pathogenesis of L-TRP EMS include clinical biochemical studies of L-TRP metabolism in patients who had developed the syndrome compared to controls; development of the first animal model of the syndrome in LEW/N rats; *in vitro* studies of pharmacologic effects and receptor binding characteristics of the synthetic contaminants; effects of the impure L-TRP and synthetic contaminants on CRH mRNA and other aspects of HPA axis function. These studies stemmed from my initial studies of L-TRP metabolism (N.Engl.J. Med., 1980) in patients who had developed the identical syndrome in relation to ingestion of L-5-hydroxytryptophan (L-5-HTP). Our understanding of the pathogenesis of the syndrome has evolved from my initial hypothesis in 1980 that the biochemical abnormality associated with the syndrome (elevated plasma kynurenine) is related to an inborn error of L-TRP metabolism. Our current understanding indicates that the elevated plasma kynurenine, seen also in the L-TRP EMS patients of 1989, is secondary to exposure to an inflammatory stimulus; that inflammation in this syndrome is initiated by exposure to one or more of the L-TRP-related impurities in the implicated L-TRP; that HPA axis suppression associated with exposure to this impurity may play a role in

amplification of expression of inflammation; and that the impurity may act through a specific receptor-mediated mechanism.

Thus, taken together, these two groups of studies take full circle the initial studies of L-5-HTP related scleroderma, and illustrate the concept that a neurotransmitter-like compound may, through several overlapping and inter-related mechanisms, be associated with susceptibility to, exacerbation of or initiation of inflammatory/autoimmune disease. The overall work of our group is thus divided into these two major overlapping and inter-related projects which will be described and presented separately.

Clinical Studies: L-Tryptophan Metabolism in L-TRP EMS patients:

Our original description of an illness related to ingestion of L-5-hydroxytryptophan, and the biochemical analysis of tryptophan metabolism described in this paper published in *The New England Journal of Medicine* in 1980, has now been recognized as the first description of the L-TRP EMS syndrome which re-appeared in 1989 in relation to ingestion of L-tryptophan. In the initial 1980 5-HTP study, plasma kynurenine was elevated in the patient studied compared to controls. This led us to conclude at that time that these patients might have had an inborn error of tryptophan metabolism, pre-disposing them to development of the scleroderma-like illness.

In our initial study of tryptophan metabolism in L-TRP EMS patients in 1989 and 1990, performed in collaboration with Dr. Melvyn Heyes (NIMH) and Dr. Richard Silver (Medical University of South Carolina, see attached *New Engl. J. Med.* article, 1990), we found once again that compared to healthy controls, plasma kynurenine was elevated in the patients with L-TRP EMS. In addition, however, we determined that quinolinic acid was also elevated in these patients. This pattern of tryptophan metabolism indicated that the alteration in L-TRP metabolism was caused by inflammation, or by exposure to a material which caused inflammation (an inflammatory stimulus), rather than to an inborn error of metabolism. In this regard, if it were an inborn error of metabolism that had caused the increase in kynurenine levels, the most likely enzymatic defect would occur in the catabolic enzymes distal to kynurenine and proximal quinolinic acid in the metabolic pathway. Thus, such a defect would result in elevated kynurenine and normal or low quinolinic acid. A parallel increase in both kynurenine and quinolinic acid, on the other hand, indicates that the increased kynurenine is secondary to induction of the rate limiting enzyme in the pathway, 2,3 indoleamine dioxygenase, with resultant increase in metabolites distal to it. Since it is now known that inflammatory stimuli and mediators such as interferon gamma induce the rate-limiting enzyme in this pathway, the presence of concurrent increased kynurenine and quinolinic acid suggest the presence of interferon gamma, or exposure to a pro-inflammatory stimulus. These studies could not identify the source of the material which caused the inflammation (i.e. a pro-inflammatory component of contaminated L-TRP versus an environmental trigger), nor could they rule out the possibility that tryptophan itself or its breakdown products might also have contributed to or amplified some of the symptoms of the disease.

We also noted in this study that six of the nine patients with the syndrome either had intrinsic HPA axis hypoactivity (Addison's disease), or were taking drugs (benzodiazepines) which prior data from our group has shown suppresses the HPA axis. In light of our findings in LEW/N rats, this suggested a possible mechanism for the potency of the inflammatory response in this illness, that is, concurrent suppression of the HPA axis due to concurrent HPA axis suppressing medications, or intrinsic hyporesponsiveness. Subsequent preliminary studies by the New York State Health Department, carried out as a result of this suggestion do suggest that a possible risk factor in development of chronic disease in this syndrome is concurrent use of psychotropic agents that our group has shown chronically suppress CRH neuronal responses. Furthermore, our ability to reproduce the cardinal features of this syndrome in LEW/N rats (see

below) also suggests that a premorbid susceptibility to inflammatory disease related to a suppressed HPA axis may further contribute to the severity and intensity of the inflammation induced by the impure L-tryptophan.

Animal studies: An animal model for the L-TRP EMS syndrome:

In order to prove a cause and effect relationship between a potential toxin and a disease, the implicated material must be tested in animals, and the resulting disease defined. Results of animal studies are outlined below. LEW/N rats fed implicated L-TRP, but not those fed non-case associated L-TRP or control, developed the same pattern of inflammation and scarring in connective tissue around the muscle as had humans who had taken the implicated L-TRP. The rats fed implicated L-TRP also showed decreased hypothalamic CRH mRNA expression. Although they did not develop eosinophilia, electron microscopic studies showed increased numbers of degranulating eosinophils and mast cells in the intestinal lining in rats fed implicated L-TRP compared to controls. The implicated and non-implicated L-TRP were fully characterized both epidemiologically and chemically by the CDC and FDA, and provided to us blind. These studies, carried out in collaboration with the FDA and CDC, were published in the *Journal of Clinical Investigation* in 1990 (attached), and provided the first definitive animal model evidence that the implicated L-TRP caused the syndrome. In addition to being of great public health value, these studies were also critical in defining the implicated impurities which should be further investigated as the prime trigger in the syndrome, and whose mechanism of action should be more fully defined.

Significance of Research on L-TRP EMS:

Clinical Studies:

We initiated work in this area with the original description of an illness related to ingestion of L-5-hydroxytryptophan. This study, and the biochemical analysis of tryptophan metabolism described in this paper published in *The New England Journal of Medicine* in 1980, has now been recognized as the first description of the syndrome which re-appeared in 1989 in relation to ingestion of L-tryptophan (1). This potentially fatal illness affecting over 1500 individuals was recently identified as a public health emergency by the CDC and FDA. This syndrome tends to occur in patients who use L-tryptophan to correct sleep disturbances in the context of depressive disorder. The patients present with fever, eosinophilia, myalgias, myositis, scleroderma-like skin fibrosis, fasciitis and neuropathies, including Guillain-Barre syndrome.

By the time the first reports of L-TRP-EMS had reached the CDC, FDA and the lay press, we were poised to advance new models for understanding fundamental mechanisms underlying the etiology and pathogenesis of this inflammatory disease. Indeed, we had already completed a biochemical study of a series of L-TRP-EMS patients and matched controls at the time that the epidemic was recognized, and it was this study which was published in *The New England Journal of Medicine* in 1990, as one of the first descriptions and the only biochemical analysis of the syndrome at that time (19).

In this first comprehensive study of the L-TRP-EMS in humans, published in *The New England Journal of Medicine*, 9 patients taking L-tryptophan were described (19), who presented with an illness almost identical to the one described by us in 1980 (1). In these subjects, we found once again, that compared to controls, affected patients showed elevation of plasma kynurenine. In addition, plasma quinolinic acid was elevated in parallel with the elevated kynurenine, in the context of normal tryptophan kinetics after tryptophan loading. These biochemical data suggested a pattern of tryptophan metabolites consistent

with activation of the enzyme indoleamine 2,3-dioxygenase (IDO). Because IDO can be activated by inflammatory triggers, these findings were consistent with initiation of the syndrome by an inflammatory stimulus, such as a contaminant in the L-tryptophan (L-TRP) preparation. Although at that time this study could not prove that the source of the inflammatory trigger was a contaminant in the implicated L-TRP, the study did finally elucidate the biochemical mechanism of the elevated plasma kynurenine which we had found in the syndrome now, and ten years earlier, and ruled out the initial hypothesis that it could be related to an inborn error of metabolism. Of note, six of the nine patients studied were also receiving drugs which we have shown suppress the hypothalamic-pituitary-adrenal (HPA) axis, or had intrinsic suppression of the HPA axis. These data are relevant to our related interest of demonstrating the role of the central nervous system in conferring susceptibility to inflammatory disease, in which deficient responsiveness of corticotropin releasing hormone (CRH) neurons to inflammatory mediators and serotonin agonists was associated with deficient pituitary-adrenal counter-regulation of the immune response via the glucocorticoids. Parenthetically, the first 5-HTP patient that we studied in 1980 was also taking an agent that we have shown is a potent suppressor of the central component of the pituitary-adrenal axis (clonazepam).

In light of these observations and the association we had recently described between hypothalamic-pituitary-adrenal axis hypo-responsiveness and susceptibility to inflammatory disease in the Lewis (LEW/N) rat, we postulated that patients with L-TRP EMS developed the syndrome because of a confluence of three factors. The first was exposure to an environmental inflammatory trigger, which could be the contaminant (s) in the L-TRP preparation. The direct testing of this hypothesis has now become possible because of our development of an animal model of L-TRP-EMS, to be described below. We postulated that a second factor conferring susceptibility to the development of L-TRP-EMS might be an intrinsic or pharmacologic suppression of the HPA axis by drugs such as benzodiazepines. Finally, we postulated, with others, that the well known effects of tryptophan and its metabolites on fibroblast proliferation, vascular permeability, vasospasm and neurotoxicity, could also contribute to the pathogenesis of the syndrome.

Development of an animal model of the EMS:

Our model of a central nervous system (CNS) role in the LEW/N rat's susceptibility to inflammatory disease proved relevant to our work with L-TRP-EMS and led to the first and only animal model of this illness. Hence, we recently published in the Journal of Clinical Investigation, that in the LEW/N rat, implicated (case-associated) L-TRP, but not pure USP grade, non-case associated L-TRP or vehicle control, was associated with development of many of the specific pathologic changes in muscle and fascia characteristic of L-TRP-EMS, in the absence of development of eosinophilia (20). We also found that ingestion of implicated L-tryptophan was associated with suppression of corticotropin releasing hormone gene expression in the paraventricular nucleus of the hypothalamus, in association with a fall in plasma corticosterone levels. Hence, these data suggested that intrinsic suppression of the hypothalamic-pituitary-adrenal axis in the LEW/N rat, coupled with a contaminant induced suppression of the corticotropin releasing hormone neuron, might have led to the expression of an eosinophilia myalgia-like syndrome in this animal model. These data not only reflect the first clear-cut evidence that contaminated L-TRP triggers the syndrome, but also provides the means to identify the role and mechanism of specific purified and synthetic contaminants in triggering the syndrome. It also provides the means to identify host factors pre-disposing certain individuals to develop the syndrome, to identify the role of the hypothalamic-pituitary-adrenal axis in susceptibility to the syndrome, to define the role of eosinophils in amplifying the syndrome, and to evaluate

new approaches to treatment of the syndrome. To further place this accomplishment in perspective, it should be noted that the toxic oil syndrome, which occurred in Spain in 1980, and affected 20,000 patients, produced a syndrome almost identical to L-TRP-EMS and resulted in thousands of deaths, and that efforts for over a decade to develop an animal model for this disorder failed. It was application of our concepts regarding the susceptibility of the LEW/N rat to the development of inflammatory disease that was essential to this accomplishment.

This work takes full circle our initial observation and description of the syndrome in the New England Journal of Medicine in 1980, which is recognized by the scientific community to be the first description of this syndrome in the scientific literature. The current series of studies define the full clinical spectrum of the EMS syndrome; define L-TRP biochemistry in the syndrome and show that the difference in L-TRP metabolism in EMS patients is secondary to inflammation or to an inflammatory stimulus rather than to an inborn error of metabolism; it suggests that an important host factor in susceptibility to the syndrome is a suppressed HPA axis; it establishes the role of contaminant(s) as the etiological trigger in the syndrome; it establishes an animal model in which to further delineate the complex cellular and biochemical mechanisms active in the pathogenesis of the syndrome; it establishes an animal model which further supports the role of the importance of HPA axis suppression in development of the syndrome; it provides an animal model in which specific contaminants can be tested and chemical structures capable of inducing this and similar syndromes can be defined; it provides an animal model which can be used for testing new approaches for the therapy of the syndrome.

In vitro effects of the tetrahydro b carboline on fetal spinal cord neuronal cell survival.

These studies provide an in vitro model through which the specific molecular mechanisms of induction of a fibrosing syndrome by a neurotransmitter molecule may be defined, and through which the target cell, the target receptor and the cytokine(s) signals induced by this compound may be defined. It provides an in vitro system in which the biological effects of other potentially related molecules may be tested. It provides a basis for understanding some of the roles of IL-1 in the developing central nervous system, and its potential pathologic effects when chemically induced.

In summary, these studies provide the cornerstone of proof that impure L-TRP caused the L-TRP EMS syndrome, and that at least one of the impurities (EBT) and its tetrahydro b carboline breakdown product caused one major pathologic feature of the syndrome (fascial thickening). In addition to addressing the public health urgency of documenting the cause and effect relationship between the impure L-TRP and development of the syndrome, these studies provide an animal and in vitro system for study of the mechanisms of induction of an autoimmune/inflammatory disease by a well defined group of compounds structurally related to neurotransmitters.

FUTURE DIRECTIONS

Current preliminary basic studies focus on determining the mechanism by which the L-TRP impurity, 1,1'-ethylidenebis(tryptophan (EBT), and its breakdown product 1S,3S tetrahydro b carboline 3 carboxylic acid (SbC) causes inflammation and affects behavior and other aspects of central nervous system function. Since animal studies have indicated that CRH may be further suppressed in rats exposed to implicated L-TRP, current and future clinical studies focus on

establishing the functional responsiveness of the HPA axis in patients during various phases of L-TRP EMS, using HPA axis studies outlined in SECTION I, HPA Axis, and will also address other aspects of the syndrome that have evolved in the chronic phase.

Basic studies:

Basic studies will focus on testing synthetic implicated impurities, and other synthetic related compounds, including the isomer of SbC, 1R,3S tetrahydro b carboline 3 carboxylic acid (RbC), in *in vivo* animal pathology and behavioral studies, and in *in vitro* cell culture studies. In collaboration with the FDA, we are also testing synthetic analogs of these compounds for their agonist/antagonist properties. The primary aim of these future studies is (1) to develop a specific antagonist(s) to SbC, which could be applied to future treatment of such syndromes, and (2) to identify and clone the receptor to which SbC binds, since this receptor and ligands which bind to it may prove critical in the initiation of a variety of inflammatory, fibrotic syndromes. In that these compounds are structurally related to neurotransmitter-like compounds, and are synthesized endogenously in a variety of tissues, they may represent a class of compounds, related to neurotransmitters, which can induce inflammatory, fibrotic disease and behavioral alterations by binding to a specific endogenous receptor.

Our future basic studies are outlined below, grouped as *in vivo* animal studies, functional *in vitro* studies, receptor binding studies, and receptor cloning studies.

(1) *In vivo* animal studies:

Pathology studies: Preliminary results of current studies directed at testing the effects of synthetic EBT in Lewis rats indicate that orally ingested EBT is associated with fascial thickening in these rats, as well as with microangiopathic changes resembling those of the human syndrome, but that all L-TRP is associated with significant fibrosis in the pancreas. This finding is of critical public health value since it reflects on the safety of not only impure, but also "pure" L-TRP, and indicates that this material should be closely monitored if ingested for therapeutic purposes.

Future directions include more extensive time course and dose response studies with oral EBT in LEW/N rats, as well as similar studies using intra-peritoneal SbC. Infiltrating cells and endothelial cells in lesions in affected tissues from all these studies will be further characterized, and sub-types of circulating peripheral blood mononuclear cells characterized and quantitated, using monoclonal antibodies, immunohistochemistry and fluorescence activated cell sorter (FACS) analysis.

HPA axis studies: Our initial studies indicate that LEW/N rats treated chronically for 38 days with either implicated S.D. L-TRP or synthetic EBT express significantly lower paraventricular hypothalamic CRH mRNA compared to LEW/N rats fed vehicle control or pure L-TRP. This is associated with a trend to lower plasma corticosterone. This finding is of potential pathogenic importance, since although this effect could not in itself have caused the inflammatory response, it could have amplified inflammation by suppressing the HPA axis response of exposed individuals.

Future directions therefore will focus on determining whether or not this effect is a direct effect of SbC or EBT, or whether it is a non-specific effect of inflammation in these animals. Studies will include time course studies (8 hours, 24 hours, 1 week, 2 weeks, 6 weeks) of paraventricular hypothalamic CRH mRNA expression in LEW/N rats fed synthetic EBT, vehicle control, or pure or implicated L-TRP. In conjunction with these *in situ* hybridization studies, plasma corticosterone and ACTH will be measured by radioimmunoassay (R.I.A.), and adrenal

and thymic weights will be determined as indicators of chronic corticosteroid levels in these rats. An additional set of control animals for *in situ* hybridization studies will be LEW/N rats sacrificed 14 days after treatment with adjuvant, as a chronically inflamed control group, since (as noted in Section I on the HPA Axis) inflammation itself could potentially alter hypothalamic CRH expression. In addition, in order to define the differential HPA axis responses of these strains to these substances, acute 1 hour studies will be performed using synthetic impurities, in LEW/N and F344/N rats. In conjunction with these studies, *in vitro* hypothalamic explant studies will be performed using LEW/N, F344/N and HSD hypothalami, in order to define the effect of these substances on CRH secretion.

Behavioral studies: Preliminary studies in rats using the Vogel test, indicate that the racemic mixture of SbC and RbC has potent anxiolytic activity. This effect will be confirmed and further defined, using full dose response studies with the pure isomers, SbC and RbC, as well as with EBT and pure L-tryptophan. This finding is important, since, together with the other studies outlined here, it would provide evidence that a single compound might produce anxiolytic, behavior-modifying effects in one system, while initiating inflammatory disease in another. Future directions in these studies include evaluation of these compounds as well as other synthetic analogs in additional behavioral paradigms, including the acoustic and tactile startle test, open field and Morris water maze to define the anxiolytic and cognitive impairing characteristics of these compounds.

(2) Functional *in vitro* studies:

We are currently testing the effects of synthetic impurities in a variety of *in vitro* cell culture systems. Read-outs include cell survival; cell proliferation, as determined by [³H]thymidine uptake and [³H]thymidine incorporation into DNA; protein content (Biorad method); and Northern blot analysis quantitation of cytokine mRNA expression, including IL-1a, IL-1b and IL-6 mRNA. Taken together, these studies address the molecular mechanisms by which the specific synthetic impurities induce a variety of clinical features of L-TRP EMS, including Guillain-Barre syndrome (ascending polyneuropathy), fibrosis and inflammation.

***In vitro* murine spinal cord neuronal cell culture studies:** Preliminary experiments, performed in collaboration with Dr. Douglas Brenneman, (NICHD), in fetal murine spinal cord neuronal cultures, indicate that the S isomer of the tetrahydro b carboline (SbC) breakdown product of EBT, but not the R isomer (RbC) or EBT itself, reverses neuronal cell death in immature cultures in a dose related manner, but is toxic to mature spinal cord cultures. The stereospecific, dose-related and compound-specific nature of this effect suggests a receptor-mediated mechanism.

Since neuronal cell survival in these cultures is related to production by accessory cells (glial cells and microglia) of a variety of growth factors, particularly IL-1a, but is not dependent on nerve growth factor (NGF), the effect of SbC on induction of IL-1a will be evaluated in cultures treated with SbC. IL-1a expression will be quantitated by Northern blot analysis, and by evaluation of the effects of specific neutralizing IL-1a antibody, and anti-IL-1 receptor antibody on the SbC cell survival effect. Full-dose response studies will be performed using SbC and these antibodies. The presence of IL-6 in these cultures will also be determined by Northern blot analysis.

***In vitro* macrophage studies:** Since survival of fetal spinal cord neuronal cells in culture depends on production of a variety of growth factors by accessory cells, including microglia, and since microglia are either derived from or related to bone marrow macrophages, the

effects of SbC, RbC and EBT will be tested on macrophages in culture. Since macrophages are also the prime source of peripheral cytokines which drive inflammation, fibroblast proliferation and eosinophilia, these studies will be critical for identifying a link between the neuronal effects of synthetic contaminants and the peripheral inflammatory and fibrotic effects. Murine macrophage cell lines, developed and characterized for inducibility of cytotoxic activity in response to inflammatory stimuli, will be used (kindly provided by Dr. Luigi Varesio, NCI). The effects of the synthetic contaminants on expression of cytokine mRNA, including IL-1a, IL-1b and IL-6 mRNA, will be evaluated by Northern blot analysis.

***In vitro* fibroblast studies:** Since one of the most characteristic features of L-TRP EMS, both in humans and in the LEW/N rat, is fibrosis, direct studies testing the effects of synthetic contaminants on murine fibroblasts in culture (3T3 cells) are under way. Cell proliferation will be evaluated by [³H]thymidine uptake and DNA incorporation, and collagen and collagenase production will be determined by Northern blot analysis.

(3) Receptor Binding and Autoradiography Studies:

Preliminary functional studies in two *in vitro* systems (fetal spinal cord neuronal cultures, and fetal liver cultures) showing a dose-related, compound, and stereospecific effect, indicate that SbC may be acting through a specific receptor. Identification and characterization of this receptor will have direct relevance for not only understanding the pathogenesis of L-TRP EMS, but also for understanding the pathogenesis of alcoholic cirrhosis. These studies will be carried out in membrane binding studies using [³H] SbC as the radioligand, as well as with competitive binding studies using unlabelled SbC to displace a selection of radioligands chosen on the basis of the structural relatedness of these compounds to SbC. Autoradiographic binding studies will also be performed, using [³H] SbC in central nervous system tissue sections.

(4) Potential agonist/antagonist studies using newly synthesized compounds:

These studies are being performed in collaboration with Dr. Samuel Page, Chief, Natural Products and Instrumentation Branch, FDA, who has synthesized a series of compounds structurally related to SbC. These compounds will be tested in the above described functional *in vitro* cell culture systems, and in the *in vivo* behavioral paradigms, as well as in receptor binding assay systems, to determine their agonist/antagonist properties. Full-dose response and time course studies will be performed, using these synthetic compounds alone, or in combination with optimal concentrations of SbC, and SbC alone as positive controls. If a specific antagonist is found, it may be useful in treatment of L-TRP EMS, or related syndromes.

Clinical Studies:

Three lines of indirect data suggest that hypoactivity of the HPA axis may contribute to the susceptibility to the development of the EMS. First, the majority of patients in our original NEJM study were taking agents known to suppress the hypothalamic component of the pituitary-adrenal axis; second, preliminary data suggest that a TRP impurity is capable of decreasing the expression of CRH mRNA in the PVN; and third, we have been able to develop an animal model of the EMS in the LEW/N rat (though we have not yet shown that other species are not equally susceptible). Hence, one of our principal clinical goals is to conduct a thorough clinical work-up of the HPA axis in patients with the EMS to carefully evaluate for the presence of a subtle central adrenal insufficiency. This work-up has been outlined in the preceding report.

Secondary goals of our clinical studies will include definition of the nature and extent of the memory loss of which these patients complain and assessment of possible corollary biological abnormalities that may be relevant to this memory loss, such as the levels of quinolinic acid in the CSF. Because epidemiological studies (New York State Health Dept.) indicate that insomnia may be an important risk factor for development of chronic disease, we shall conduct detailed sleep studies, including assessment of the presence or absence of frank sleep apnea or hypopnea. An inherent difficulty in these studies is the fact that the chronic disease itself may alter sleep patterns, and thus make determination of an underlying association between sleep pattern and development of chronic disease difficult to determine.

In order to determine whether a group of diseases similar to L-TRP EMS exists in relation to a family of related compounds, patients who develop similar symptoms in relation to related compounds, such as L-5-HTP, will be fully evaluated, both with an extent-of-disease work up for inflammation, fasciitis and eosinophilia, as well as with HPA axis studies. These studies will be performed in collaboration with Dr. Samuel Page at the FDA, who will fully chemically characterize ingested compounds, initially by HPLC, and subsequently by mass spectrometry.

Summary of Hypothetical Causes of L-TRP EMS:

Based on these studies, our current working hypothesis for the etiology and pathogenesis of L-TRP EMS is summarized below:

- One or several components of implicated L-TRP may trigger acute inflammation.
- L-TRP itself and some of its metabolites (eg quinolinic acid) may contribute to some of the features of the illness, although alone it does not cause the syndrome.
- The acid breakdown product of one of the impurities of the case-associated L-TRP preparation affects cell survival and proliferation in a number of systems in a dose-related, stereospecific and compound-specific manner, suggesting that it acts through a receptor-mediated mechanism.
- Products released from eosinophils may also contribute to some aspects of the illness.
- The acute inflammatory process, through unknown mechanisms, may set into motion a self-perpetuating immune disease in susceptible individuals.
- The implicated L-TRP or its impurities may exacerbate inflammation by suppressing the hypothalamic-pituitary-adrenal axis response.
- Taken together these findings have potential relevance not only to L-TRP EMS but also to our understanding of the mechanisms through which many other inflammatory and fibrotic diseases, such as scleroderma, may be induced.

Publications:

DeSchryver-Kecsckemeti K, Gramlich TL, Crofford LJ, Rader JI, Page SW, Needham LL, Hill RH Jr, Sternberg EM. Mast cell and eosinophil infiltration in intestinal mucosa of Lewis rats treated with L-tryptophan. *Mod Pathol* 1991;4(3):354-57.

Hertzman PA, Maddox GL, Sternberg EM, Heyes MP, Mefford IN, Kephart GMM, Gleich GJ. Repeated coronary artery spasm in a patients with the eosinophilia myalgia syndrome: Deposition of eosinophil granule major basic protein in the heart. *J Am Med Assoc*, 1992.

Sternberg EM, Testimony to the 101st Congress of the United States, House of Representatives, Human Resources and Intergovernmental Relations Subcommittee of the Committee on Government Operations, Hearing on Issues Related to the Federal Government's Regulation of L-tryptophan Eosinophilia Myalgia Syndrome. In: *Congressional Record*, 1991.

Sternberg EM. Interdisciplinary lines of investigation and implications for the pathogenesis of the L-tryptophan eosinophilia myalgia syndrome. In: Toxic Oil Syndrome and Eosinophilia-Myalgia Syndrome: Pursuing Parallels in Pathogenesis, Report on a WHO Meeting, Washington, DC, 8-10 May, 1991

The following table shows the results of the experiments conducted on the effect of the temperature of the water on the rate of the reaction between hydrogen peroxide and potassium permanganate. The experiments were conducted at different temperatures, and the rate of the reaction was measured by the volume of oxygen gas evolved.

Temperature (°C)	Volume of Oxygen (ml)	Time (min)	Rate (ml/min)
10	10	10	1.0
20	20	10	2.0
30	30	10	3.0
40	40	10	4.0
50	50	10	5.0
60	60	10	6.0
70	70	10	7.0
80	80	10	8.0
90	90	10	9.0
100	100	10	10.0

The results of the experiments show that the rate of the reaction increases with the temperature of the water. This is because the rate of a chemical reaction increases with the temperature. The rate of the reaction is directly proportional to the temperature of the water.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02402-02 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) Antidepressant effects of light in winter seasonal affective disorder		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: N. Rosenthal Senior Clinical Investigator CPB/NIMH (On Site; Overall PI - Martin Teicher, M.D., McLean Hospital) Others: D. A. Oren Senior Clinical Investigator CPB/NIMH P. Schwartz Clinical Associate CPB/NIMH C. Brown Psychologist CPB/NIMH C. Luetke Research Assistant CPB/NIMH		
COOPERATING UNITS (if any) McLean Hospital, Harvard Medical School.		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION Environmental Psychiatry		
INSTITUTE AND LOCATION NIMH Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Previous studies of the efficacy of <u>light therapy</u> for winter <u>seasonal affective disorder</u> (SAD) have utilized light boxes, which, though effective and safe, are cumbersome and, at times, inconvenient. A head-mounted, <u>portable light delivery system</u> has been invented and developed by researchers in the Intramural Program of the NIMH and at Jefferson Medical College in an attempt to find a more convenient light treatment source. This past winter, we participated in the third multicenter clinical trial of this device in as many years. The latest study was not performed as part of a CRADA with Bio-Brite, Inc. of Bethesda, MD, the manufacturer of the <u>visor</u>, though the company did provide the visors without charge.</p> <p>In the two earlier studies, we found no difference between visors of widely varying intensities (from 60 to 5000 lux). Although all treatments appeared to be fairly effective, the question remained as to whether this effect was due solely to the placebo effect or to specific effects as well. To address this question, we undertook a third study, in which a very dim (30 lux) red visor, which we predicted would function only as a <u>placebo</u>, was compared to a somewhat brighter (600 lux) white visor in a parallel design in 57 patients with SAD from 2 centers (NIMH, Bethesda, MD and McLean Hospital, Belmont, MA) were assigned randomly to treatments with either a 30-lux red visor or a 600-lux white visor. Treatments were administered for 30 minutes each morning for 2 weeks. The overall response rate for both visors, using stringent response criteria, was approximately 40%. Thus, our prediction that the dimmer visor would be more effective than the brighter one was not confirmed. Possible explanations for this finding are discussed.</p>		

Antidepressant Effects of Light in Seasonal Affective Disorder

Since we first showed that bright light could be used as an effective antidepressant in the treatment of winter seasonal affective disorder (SAD), we have performed a number of studies to investigate those formal properties of light necessary for its therapeutic effect, as well as optimal methods of light delivery. Thus, we showed that the antidepressant effect is mediated via the eyes rather than the skin, that it depends upon the intensity of the light source, that an antidepressant response can be observed when light is administered at various times during the day, and that green light is more effective than red light.

More recently, we have addressed the practical problem of trying to make light therapy more convenient. The traditional method of administering light therapy by using light boxes, which are safe and effective, is cumbersome because these devices are not easily transported. To overcome this inconvenience, we have developed a portable, battery-powered, head-mounted "light visor" in conjunction with researchers at Jefferson Medical College. We have previously tested this device in two separate multicenter studies. In addition to the advantage of portability and convenience, the light from the visor remains in fixed relation to the wearer's eyes during light therapy sessions whereas, when light therapy is administered from a light box, small movements of the head can result in large variations in the amount of light reaching the eyes.

The light visor was licensed and manufactured by the Bio-Brite Company, who provided visors and funding for two earlier studies by means of a Collaborative Research and Development Agreement (CRADA) between the company and the NIMH. The study performed this past year was not done as part of a CRADA with Bio-Brite, though the company provided the visors without charge.

The light visor resembles a baseball cap, from which two incandescent, filtered light sources direct light toward the eyes. In the first visor study, conducted during the winter of 1989-90 at three centers (Bethesda, MD; Nashua, NH; and Seattle, WA), fifty-five SAD patients participated in a parallel design study in which we compared visors of two intensities of white light: 500 lux and 5000 lux. On the basis of earlier light box studies, in which a direct relationship between intensity and efficacy was noted, we hypothesized that the brighter (5000 lux) visor would be more effective than the dimmer (500 lux) one. Patients were treated for either 30 or 60 minutes on arising in the morning. Contrary to our prediction, the brighter visor was not more effective than the dimmer one. Paradoxically, when response rates were compared using stringent response criteria, the dimmer visor was almost superior to the brighter one. Response rates for the two visors were 56% and 27% respectively, the former value comparing favorably with response rates found in some earlier light box studies.

In an attempt to clarify and elaborate on these unexpected findings, we participated in a five-center study, co-ordinated by Dr. Russell Joffe at the Clarke Institute, Toronto. Other centers involved, apart from our own, were University of British Columbia, Vancouver; University of Utah, Salt Lake City; and McLean Hospital, Harvard University, Belmont, Massachusetts. We tested three different intensities of white light: 3200 lux, 600 lux and 60 lux. Once again, we hypothesized some direct relationship between intensity and efficacy and predicted that the dimmest visor would be the least effective. A total of 105 subjects were studied, 30 of these at the NIMH. Again, we found no difference between the effects of the three visors. Stringently measured response rates for the 60 lux, 600 lux and 3200 lux visors were 45%, 50% and 54% respectively.

We recognized that there were at least two alternative explanations for the paradoxical findings of the previous years. First, the antidepressant response might have been due entirely to placebo effects. Second, all visors might have exceeded the threshold for a true biological response to light therapy. This past year we undertook a third study in this series in an attempt to dissect apart these two possibilities. Arguing that perhaps the dimmest of the visors in the previous studies were not dim enough, we tested an even dimmer visor (30 lux), emitting red light, which has been shown to be relatively inert biologically, against a brighter (600 lux) white visor. The study was performed at two sites, our own and McLean Hospital, a Harvard University affiliate, and was co-ordinated out of the latter site.

Project Description and Methods:

During the winter of 1991-92, 57 winter SAD patients at 2 centers (Bethesda, MD; and McLean Hospital, a Harvard University affiliate) participated in a parallel design study comparing visors of two intensities and different spectra: 30 lux red light and 600 lux white light. After a baseline week in which patients kept to a regular sleep-wake schedule, those who remained severely enough depressed were randomly assigned to one of the two visor types for one-half hour each morning for two weeks. This was followed by one week of withdrawal. Mood was evaluated on a weekly basis by raters blind to the patients' treatment status. In order to minimize the confounding effects of ambient light, we asked patients to wear dark wrap-around goggles (3% light transmittance) whenever they were outdoors during the four study weeks.

Findings to date:

Fifty seven subjects were studied at the two centers; 30 of these at the NIMH. There was no difference between the effects of the two visors, regardless of whether response was judged by reduction in depression ratings or by determination of responder rates according to established criteria. Stringent response rates for the 30 lux red and 600 lux white visors were 41% and 39% respectively. There was no difference in response rates between centers.

Significance to Biomedical Research and to the Program of the Institute:

We are still unable to say with certainty why visors of widely differing intensities yielded no difference in response rates, both in the present study and in the previous two studies. The first possible explanation for these observations is that they are due entirely to placebo or non-specific effects. Problems with this explanation are that the response rates noted in these visor studies exceeded those reported for dim light boxes or placebo pills used in earlier studies. Instead, the response rates obtained with the visor resembled those reported for bright (2500 lux) light boxes in some earlier studies. It is possible that the placebo effect was more powerful in the visor studies because the visor itself might be a more plausible placebo or because of the growing popularity of light therapy.

A second possible explanation is that the light visor might be a moderately effective source of light therapy with unusual photic properties. For example, it is possible that when the light source is close to the eyes, certain regulatory mechanisms, such as squinting, pupillary constriction or retinal responses, come into play to gate the amount of light entering the eyes. The efficacy of the 30 lux red visor is still difficult to credit, however, as a source of truly effective light therapy.

Finally, the light visor might be working by mechanisms other than placebo or its photic properties. For example, simply having patients wake up and undertake a certain activity at a regular time every day might have a salutary effect on their circadian rhythms and therefore on their mood.

Proposed Course:

We plan no further studies with the light visor at this time. Future studies will focus instead on ways of enhancing the efficacy of conventional light treatment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02206-08 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (<i>80 Characters or less. Title must fit on line between the borders.</i>) Neurobiology of Seasonal Affective Disorder (SAD) and Light Therapy		
PRINCIPAL INVESTIGATOR (<i>List other professional personnel below the Principal Investigator.</i>) (Name, title, laboratory, and institute affiliation)		
PI:	N.E. Rosenthal	Chief of Environmental Psychiatry CPB/NIMH
Others:	D.A. Oren	Senior Clinical Investigator CPB/NIMH
	D.E. Moul	Senior Clinical Investigator CPB/NIMH
	P. Schwartz	Clinical Associate CPB/NIMH
	C. Brown	Program Coordinator CPB/NIMH
	F. Meyers	Nurse CPB/NIMH
	D. Garcia-Borreguero	Visiting Fellow CPB/NIMH
COOPERATING UNITS (<i>if any</i>) Research Biology Unit, FDA Laboratory of Neuroscience, NIDDK		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION Environmental Psychiatry		
INSTITUTE AND LOCATION NIMH Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	2	PROFESSIONAL: 1 OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (<i>Use standard unreduced type. Do not exceed the space provided.</i>) <p>Although repeated studies have established that <u>bright light</u> is an effective treatment for <u>SAD</u>, the mechanism of its actions remains unknown, as do the fundamental biological abnormalities responsible for the syndrome. There is ample reason to believe that brain serotonergic systems may be involved in these processes.</p> <p>In the past, we undertook preliminary studies to investigate whether SAD patients would respond differently from normal controls to infusions of the somewhat selective <u>serotonin</u> agonist, <u>m-chlorophenylpiperazine (m-CPP)</u>. We found that when untreated during the <u>winter</u> months, SAD patients reported being activated to a greater degree than normal controls following the infusion. Effective light treatment or the advent of summer were associated with a normalization of this activation response. Our earlier studies suffered from certain methodological deficiencies. In the present study we have attempted to obviate these deficiencies by increasing our sample size, including a placebo infusion condition and randomly ordering treated, "on lights," and untreated, "off lights," conditions. Seventeen patients who met criteria for winter SAD and 15 healthy controls underwent infusions of either m-CPP or placebo on separate days in both "off lights" and "on-lights" conditions. The "on-lights" condition consisted of 2 weeks of daily treatments with 10,000 lux of white light for 45 minutes in the morning and 45 minutes in the evening; the "off-lights" condition consisted of at least two weeks without any light treatment. Infusions consisted of either 0.08 mg/kg of m-CPP in normal saline or the saline alone. At baseline and for 90 minutes following the infusion, we measured behavior by means of a 24-item NIMH self-rating scale, body temperature, and drew blood for hormonal measurements.</p> <p>As we had found previously, the <u>"activation-euphoria"</u> subscore increased significantly in SAD patients during the "off lights" condition, as compared with the "on-lights" condition or with the normal controls under either condition ($F=4.7$; $df = 3, 90$; Greenhouse-Geisser corrected $P<.05$). Increase in activation following m-CPP infusions in patients in the "off-lights" condition correlated significantly with antidepressant response to light, as measured by the Hamilton Depression Rating Scale. We thus replicated and strengthened our earlier finding. Serotonergic abnormalities appear to related to the pathophysiology of SAD and its response to light. Further studies along</p>		

Neurobiology of Seasonal Affective Disorder (SAD) and Light Therapy

Although repeated studies have established that bright light is an effective treatment for SAD, the mechanism of its therapeutic actions remains unknown, as do the fundamental biological abnormalities responsible for the syndrome. In the past we have explored the possibility that abnormalities in melatonin secretion or an abnormal response to this hormone might be responsible for the symptoms of SAD; and that light therapy might exert its antidepressant effects by modulating melatonin secretion. Several tests of this hypothesis resulted in predominantly negative findings. Subsequently we evaluated brain dopaminergic systems in SAD but failed to find evidence that these are abnormal in this condition. More recently we have attempted to elucidate the pathophysiology of SAD and the mechanism of action of light therapy by exploring two lines of research: the eye and neural pathways connecting the eye to the brain; and brain serotonergic systems. Explorations of the eye and visual pathways are outlined in a separate report (# Z01 MH 02611 - 01 CP). In this report, I will discuss our exploration of brain serotonergic systems.

There is ample reason to believe that brain serotonergic systems are disturbed in SAD. Patients with this condition crave carbohydrates and report feeling activated when they consume sweets and starches. Carbohydrate-rich meals have been shown to increase brain serotonin synthesis in animals, an effect that has been postulated to occur in humans as well. In an earlier study, we fed patients meals rich in either carbohydrate or protein and found, as we had predicted, that carbohydrate-rich meals activated SAD patients but sedated normal controls. In a study, undertaken at M.I.T., SAD patients were found to respond to the serotonin agonist *d*-fenfluramine to a greater degree than to placebo. In a post-mortem study of human brains, derived from individuals who died at different times of the year, hypothalamic serotonin content was found to drop during the winter months. This could be the physiological basis for why certain vulnerable individuals become depressed during the winter.

In the past, we undertook preliminary studies to investigate whether SAD patients would respond differently from normal controls to infusions of the somewhat selective serotonin agonist, *m*-chlorophenylpiperazine (*m*-CPP). We found that when untreated during the winter months, SAD patients reported being activated to a greater degree than normal controls following infusions of *m*-CPP. Effective light treatment or the advent of summer were associated with a normalization of this activation response. In addition, SAD patients showed exaggerated cortisol and prolactin responses to *m*-CPP, providing further evidence of serotonergic abnormalities in this condition insofar as the secretion of both of these hormones is regulated in part by serotonin. These hormonal responses were decreased by light treatment in both patients and normal controls.

Although our earlier *m*-CPP studies of SAD strongly suggested abnormalities in brain serotonergic systems, which are normalized by light therapy, they suffered from certain methodological deficiencies. First, the number of subjects studied was relatively small. Second, we used no placebo infusions. Thus aberrant behavioral and hormonal responses could be interpreted as representing non-specific reactions in a pathological population. Finally, all patients were studied first in the untreated and subsequently in the light-treated condition. An ordering effect might thus have confounded the apparent effects of light therapy.

Project Description and Methods:

The object of the study was to evaluate brain serotonergic systems in untreated and light-treated patients with seasonal affective disorder (SAD) and normal controls. Seventeen patients who met criteria for winter SAD and 15 healthy controls underwent infusions of either *m*-CPP or placebo on separate days in both untreated, "off lights," and light-treated, "on-lights," conditions. The "on-lights" condition consisted of two weeks of daily treatments with 10,000 lux of white light for 45 minutes in the morning and 45 minutes in the evening; the "off-lights" condition consisted of at least two weeks without any light treatment. In addition, when off lights, subjects were asked to wear dark wrap-around goggles (3% transmittance) whenever they were outdoors, in order to maximize the differences between conditions. Both light treatment conditions and infusions were administered in a randomly ordered way. Before each infusion, patients slept overnight in an inpatient unit of the NIMH and lighting conditions were carefully controlled at a

low intensity during the morning of the infusion. Infusions consisted of either 0.08 mg/kg of m-CPP in normal saline or the saline alone. At baseline and for 90 minutes following the infusion, we measured behavior by means of a 24-item NIMH self-rating scale, body temperature, and drew blood for hormonal measurements.

Findings to Date:

At the time of writing, bloods are still being assayed for hormone levels and body temperature profiles are still being analyzed. This report will therefore deal only with the results of behavioral measures. Light therapy was effective and resulted in a reduction of mean patient Hamilton Depression Rating Scale (HDRS) scores from 12 to 5. Analysis of the baseline self-rating scale scores showed significant differences between patients and controls on most subscale scores. As we had found previously, the "activation-euphoria" subscore increased significantly in SAD patients following infusion of m-CPP during the "off lights" condition, as compared with the "on-lights" condition or with the normal controls under either condition ($F=4.7$; $df = 3, 90$; Greenhouse-Geisser corrected $P<.05$). There were no significant differences between the responses of patients and controls on any other self-rating subscore. Increase in activation following m-CPP infusions in patients in the "off-lights" condition correlated significantly with antidepressant response to light, as measured by the HDRS. In fact, activation following infusion was a better predictor of decrease in the HDRS total score than any individual HDRS symptom at baseline.

Significance to Biomedical Research and to the Program of the Institute:

The activating and euphoriant effects of m-CPP on untreated patients with SAD, which we have replicated in the present study, is one of the few replicated biological abnormalities described in this condition. The superiority of the methodology of the new study, namely the use of a placebo control, balanced ordering of the light treatments and the larger sample size, add strength to the finding. This behavioral response appears specific for SAD in that m-CPP has been administered to patients with several other types of psychiatric conditions without resulting in this particular response. There were no other aberrant responses to m-CPP in the SAD patients, which further defines the specificity of the response. Insofar as light treatment reversed this abnormal response, it is possible that light treatment may be acting via the same receptors that are stimulated by m-CPP. Chronic light treatment might desensitize these receptors, thereby diminishing subsequent exposure to the drug. The significant correlation between the activating effects of the drug and the antidepressant response to light, as measured by a standard depression scale, further suggests an association between these two different interventions.

Proposed Course:

The present study, along with its predecessor and data from other centers, provides strong evidence of the involvement of serotonergic pathways in SAD and its response to light therapy. Further investigations of this possible association are clearly warranted. Future studies will focus on other perturbations of serotonergic systems in SAD patients and controls to pin down more precisely the role of these systems.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02294-08 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) Antidepressant Pharmacology of the Rodent Circadian System		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Wallace C. Duncan Research Psychologist CPB/NIMH Others: Norio Ozaki Fogarty Fellow Thomas A. Wehr Chief, Clinical Psychobiology Branch		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.0	PROFESSIONAL: .75	OTHER: .25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have hypothesized that the therapeutic mechanism of antidepressant drugs depends on their effects on the circadian system. This hypothesis is being examined by testing the effects of antidepressant and neuroleptic drugs on the state of the circadian pacemaker that controls daily rhythms of motor activity, temperature and EEG sleep. </p> <p> During the past year this project has focused on two major areas. One area of investigation has been on the effects of chronic psychoactive drug treatment on regulation of brain temperature. These experiments indicate that chronic antidepressant drug treatment with <u>clorgyline</u>, <u>fluoxetine</u> or <u>lithium</u>, lowers <u>hypothalamic temperature</u>, particularly during the rest phase of the circadian cycle. In contrast, chronic treatment with the <u>neuroleptic</u> drugs <u>chlorpromazine</u> or <u>haloperidol</u> increase hypothalamic temperature. Further research is required to understand the mechanisms through which these drugs alter hypothalamic temperature. For example, it is possible that antidepressant drugs decrease the set-point of hypothalamic temperature regulation, possibly through their serotonergic properties. These drugs, through multiple effects on circulating hormones and neurotransmitters, could also alter hypothalamic temperature by changing blood flow to the hypothalamus. In depressed patients, elevated body temperature is often observed during rest, and pharmacological and non-pharmacological treatments of depression have been reported to lower body temperature. Therefore, the findings that antidepressant drugs decrease hypothalamic temperature may be important in understanding their therapeutic mechanism. </p> <p> In light of the effects of antidepressant drugs on circadian variation of hypothalamic temperature, a second area of research has been to determine the chronic effects of <u>clorgyline</u>, an MAOI which a) chronically decreases hypothalamic temperature in hamsters and b) delays the circadian pacemaker, on brain monoamines in discrete brain nuclei reported to be involved in circadian regulation of behavior and thermoregulation. These studies indicate that chronic clorgyline treatment elevates <u>serotonin</u> (5HT) levels in terminal regions of the <u>hypothalamus</u> (suprachiasmatic nucleus, paraventricular nucleus, medial preoptic area) and thalamus (lateral geniculate nucleus), and in cell bodies of the dorsal and median raphe. It also elevates <u>dopamine</u> levels in the ventral tegmental area, the caudate nucleus and the nucleus accumbens. Furthermore, in all regions except the <u>dorsal raphe</u> nucleus and the <u>suprachiasmatic nucleus</u>, clorgyline treatment eliminated the circadian rhythm of 5HT that is normally present. </p>		

Project Description

The therapeutic mechanism of antidepressant drugs may depend on their ability to alter the physiological state of the central circadian clock. Many patients receiving drug treatment experience disruption of the activity-rest cycle, or a change in the circadian rhythm of body temperature during the course of treatment. These drug effects may result from pharmacological effects on selected components of the circadian pacemaker. Our objective is to understand the effects of antidepressant drugs on the circadian pacemaker and on processes controlled by the pacemaker.

Methods

1) Experimental equipment

A description of the facility used to monitor the rodent circadian system can be found in project report Z01 MH 02294-01 CP. A description of the equipment can be found in project report Z01 MH 02294-04 CP. We record hypothalamic temperature using the Mini-Mitter telemetry system.

2) Effects of Psychoactive Drugs on the Daily Pattern of Telemetred Hypothalamic Temperature in Syrian Hamsters

Hamsters are implanted with intraperitoneal transmitters or hypothalamic brain probes in order to simultaneously monitor peritoneal or hypothalamic temperature as well as motor activity. Temperature is recorded every five minutes for 6-8 weeks. After a stable baseline interval has been recorded, hamsters are chronically treated with antidepressant or neuroleptic drugs using Alzet mini-osmotic pumps (clorgyline, saline), liquid diet (fluoxetine), chow (lithium) intramuscular injection (haloperidol decanoate, vehicle) or pellet (chlorpromazine). Data are collected and analyzed using a laboratory computer. Serum drug levels are measured in lithium, haldol, chlorpromazine and fluoxetine-treated animals. Hamsters are housed in LD 14:10 or constant darkness (DD). Some experiments utilize telemetry methods to simultaneously monitor hypothalamic and peritoneal temperature. At the end of each experiment, hamsters are euthanized and the location of the hypothalamic temperature probe is verified using histological technique.

3) Effects of Clorgyline on Circadian Variation of Monoamine Levels in Discrete Brain Regions

Hamsters are chronically treated with clorgyline as described above. At the end of treatment, hamsters are euthanized at different times of the day and brain tissue is collected, rapidly frozen and stored at -80°C. Serial 300 μ brain sections are dissected using a micropunch. Regions punched include caudate nucleus (CN), nucleus accumbens (NA), medial preoptic area (MPO), suprachiasmatic nucleus (SCN), paraventricular nucleus of the hypothalamus (PVN), ventral lateral geniculate nucleus in the vicinity of the intergeniculate leaflet (LGN), substantia nigra (SN), ventral tegmental area (VTA), dorsal raphe (DR) and median raphe (MR). 5HT and DA levels are determined by HPLC using electrochemical detection. Locations of the punches are verified using histological technique. Data are analyzed using one or two-factor analysis of variance as well as cosinor analysis.

Findings to date

1) Effects of Psychoactive Drugs on the Daily Pattern of Telemetred Body Temperature in Syrian Hamsters

a) Antidepressant vs Neuroleptic Treatment

Chronic treatment with all the antidepressant drugs studied thus far significantly decreased hypothalamic temperature. The decrease in hypothalamic temperature was greatest during the light phase. Two-factor anova indicated a significant decrease following chronic treatment with

clorgyline, ($p=.001$), lithium ($p=.01$), and fluoxetine ($p=.03$). In contrast, chronic treatment with neuroleptic drugs either increased or had negligible effects on hypothalamic temperature. Haloperidol decanoate tended to elevate hypothalamic temperature during the light phase ($p=.07$), and chlorpromazine had a brief temperature elevating effect. The relationship between serum drug levels and hypothalamic temperature is currently being examined.

b) Participation of Melatonin:

Experiments have been conducted to examine the mechanism of the temperature-decreasing effects of clorgyline. Pineal melatonin levels were measured following chronic clorgyline treatment and found to be significantly increased ($p=.0001$). In order to examine whether drug-induced increases in the level of melatonin mediated the decrease in temperature, hamsters were pinealectomized and then treated with clorgyline. However, pinealectomized hamsters responded to clorgyline treatment the same way as sham-pinealectomized control hamsters. That is, both groups of hamsters exhibited a decrease in hypothalamic temperature following clorgyline treatment. Therefore, clorgyline-induced elevation of melatonin does not mediate the decrease of hypothalamic temperature.

c) Drug Interaction with the Light-Dark Cycle

Experiments have also been conducted in constant darkness to determine if an interaction between the light-dark cycle and clorgyline treatment is associated with changes in brain temperature. Three hamsters were studied for seventeen days in LD followed by eleven days in continuous darkness. In continuous darkness, clorgyline's effect on hypothalamic temperature was unaffected during rest (when light would normally occur). In contrast, clorgyline's effect on hypothalamic temperature during activity (when darkness would normally occur) showed a decrease compared to its level measured during the light-dark cycle ($p=.007$). This preliminary experiment suggests a possible interaction between clorgyline's effects on hypothalamic temperature and the light-dark cycle. Further it suggests that light is not necessary for clorgyline's temperature-reducing effects. Additional animals will be studied to fully examine this interaction.

d) Relationship Between Hypothalamic Temperature and Food Consumption

Hamsters fed a liquid diet containing fluoxetine consumed about 10 kcal/day less food than during a pretreatment period. To examine whether limiting caloric intake was related to the decrease in hypothalamic temperature during drug treatment, untreated hamsters were restricted by 10 kcal/day. These animals also showed a decrease in hypothalamic temperature, but no significant loss of body mass. Although these results suggest fluoxetine's effects on caloric intake might cause the decrease in temperature, this interpretation is likely to be an oversimplification since fluoxetine injections (i.p.) were found to decrease brain temperature within one hour. A more likely explanation is that food restriction and fluoxetine are affecting a common, possibly serotonergic, pathway, which then mediates the decrease in brain temperature.

(2) Clorgyline Effects on Circadian Variation of Monoamine Levels in Discrete Brain Regions

In control hamsters, single factor anova indicated significant circadian variation in the level of 5HT in all regions examined (SCN, $p=.01$; PVN, $p=.05$; MPO, $p=.01$; LGN, $p=.01$; DR, $p=.006$; MR, $p=.005$). Levels of DA in CN, NA, VTA and SN did not show a significant circadian variation. In clorgyline-treated hamsters, only SCN ($p=.04$) and DR ($p=.04$) showed a significant circadian variation. Cosinor analysis confirmed a significant 5HT rhythm in SCN and DR from clorgyline-treated hamsters. Further, there was a significant phase-delay in the rhythm of 5HT measured from SCN in clorgyline-treated hamsters compared with saline hamsters. DA levels did not show a significant circadian variation during clorgyline treatment.

Two-factor analysis of variance indicated that clorgyline treatment significantly elevated 5HT levels in all regions examined (SCN, PVN, MPO, LGN, DR, and MR; $p=.0001$). DA levels were significantly elevated by clorgyline treatment in CN ($p=.004$), VTA ($p=.0001$) and NA ($p=.003$) but not in the SN. There was a significant interaction between drug treatment and time of day on 5HT levels measured from the SCN ($p=.053$) and from the MR ($p=.01$) and a trend in the DR ($p=.097$). The findings that clorgyline chronically elevates central levels of 5HT, and these levels are elevated in regions known to participate in circadian regulation and thermoregulation, strongly

suggests that this neurotransmitter participates in the effects of clorgyline on thermoregulation and on the circadian pacemaker.

Significance to Biomedical Research

In Syrian hamsters, chronic antidepressant drug treatments studied to date seem to share the common property of decreasing the level of brain temperature. We hypothesize that antidepressant drugs in general may share this property. If this hypothesis is correct, and if this effect on temperature is essential for the therapeutic effect of the drugs, these experiments may lead to the development of more specific, clinically useful antidepressant drug treatments for depression. In addition these findings may lead to the identification of novel, non-pharmacological treatments of affective disorders.

Proposed Course

We will extend these studies to tricyclic antidepressant drugs to determine if our observations can be generalized. Our current hypothesis is that elevation of central 5HT levels by antidepressant drugs mediates their effects on decreased hypothalamic temperature. Since the MAOI clorgyline induces large and sustained changes on 5HT levels in the dorsal raphe and the suprachiasmatic nucleus, and since these nuclei participate in circadian variation of temperature, it will be important to investigate antidepressant drug effects on 5HT levels in these areas. The use of selective 5HT agonists and antagonists will be used to identify 5HT receptors that are involved in the regulation of hypothalamic temperature.

A second area of interest is related to the observation that the temperature-reducing effects of these drugs is most apparent during the coexisting light and rest phases of the respective light-dark and activity-rest cycles. An unresolved issue is whether the temperature-reducing effects are more related to light (to which some hormone rhythms are coupled, e.g. melatonin,) or to rest (to which other hormone systems are coupled, e.g. corticosteroids). Our preliminary data suggests that the light-dark cycle may interact with drug treatment to affect hypothalamic temperature during the dark phase, but not during the light phase. To determine whether the temperature-reducing effects of antidepressant drugs is coupled to the rest phase, we will examine their effects in a diurnal rodent that rests during the dark phase.

Publications

Gao, B., Wehr, T.A., Duncan, W.C. (1991) Clorgyline-induced reduction in body temperature and its relationship to vigilance states in Syrian hamsters. Neuropsychopharmacology 4: 187-197.

Gao, B., Duncan, W.C., Wehr, T.A. (1992) Fluoxetine decreases brain temperature and REM sleep in Syrian hamsters. Psychopharmacology 106:321-329.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02403-02 CP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of light in HIV Patients

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: N.E. Rosenthal Chief, Section on Environmental Psychiatry, CPB/NIMH
Clinical Psychobiology Branch

Others: Charlotte Brown Psychologist CPB/NIMH
D.A. Oren Senior Clinical Investigator CPB/NIMH
P. Schwartz Clinical Associate CPB/NIMH
G. Galetto Clinical Associate MB/NIMH

COOPERATING UNITS (if any)

Medical Branch, National Cancer Institute, NIH

LAB/BRANCH

Clinical Psychology Branch

SECTION

Section on Environmental Psychiatry

INSTITUTE AND LOCATION

NIMH Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project Terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02405-06 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) Effects of Chemical Antidepressants on Body Mass and Composition in Hamsters		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation) PI: Wallace C. Duncan Research Psychologist CPB/NIMH Others: Christopher Gordon Research Physiologist, US EPA Thomas A. Wehr Chief, Clinical Psychobiology Branch		
COOPERATING UNITS (if any) Neurotoxicology Division, Health Effects Research Laboratory U.S. Environmental Protection Agency		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH Bethesda, Maryland 20892		
TOTAL MAN-YEARS: .5	PROFESSIONAL: .25	OTHER: .25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Antidepressant</u> drugs are reported to decrease the resting metabolic rate and increase body mass of depressed patients. Increased body mass may be partly related to the decrease in metabolic rate. The change in energy budgeting that follows chronic antidepressant treatment may be related to the behavior-activating effects of the drugs. This project investigates the functional (e.g. thermoregulatory) and endocrine aspects of altered body mass. </p> <p> Previously we found that chronic inhibition of type A monoamine oxidase (<u>MAO</u>) in Syrian hamsters with the antidepressant drug clorgyline a) prevents normal weight gain, b) decreases body lipid content, c), decreases oxygen and food consumption, and d) decreases the level of peritoneal and brain temperature. We also found that chronic clorgyline treatment alters <u>adrenal</u>, kidney, testis and <u>brown adipose tissue</u> (BAT) masses, suggesting that clorgyline-treatment might have significant endocrine effects. In light of these organ data, as well as reports that melatonin and corticosteroids affect thermoregulation, we examined the daily variation of cortisol, corticosterone, ACTH and melatonin in clorgyline-treated hamsters to assess their possible roles as mediators of clorgyline's effects on <u>thermoregulation</u>. Chronic clorgyline treatment was found to elevate pineal <u>melatonin</u>, serum <u>ACTH</u>, <u>cortisol</u> and <u>corticosterone</u>. The observation that chronic <u>clorgyline</u> decreases the mass of BAT and elevates levels of corticosteroids is consistent with reports that chronic corticosterone administration decreases functional activity (GDP binding) of BAT. In contrast, although melatonin is reported to activate BAT thermogenesis, elevation of melatonin levels was not associated with increased BAT mass. Thus, in Syrian hamsters, chronic antidepressant drug treatment with the MAOI clorgyline may alter thermoregulation by corticosteroid-mediated inhibition of non-shivering thermogenesis (NST). </p> <p> In collaboration with Dr. Christopher Gordon, we explored effects of clorgyline on autonomic and behavioral aspects of thermoregulation in Syrian hamsters. At cold ambient temperatures, clorgyline elevated metabolic rate and motor activity, possibly to compensate for the loss of insulative body fat and/or diminished NST. When placed on a thermal gradient for 24 hours, clorgyline-treated hamsters tended to select cooler ambient temperatures, and the <u>circadian</u> rhythm in thermal preference that is normally present was absent. Drug-treated hamsters also spent less time in the thermoneutral portion of the thermocline, suggesting that these animals' capacity to thermoregulate was less finely tuned than control animals. </p>		

Project Description

Antidepressant drug treatment is associated with changes in body mass and body temperature in depressed patients. Changes in body mass and thermoregulation are also observed in chronic drug-treated hamsters. The purpose of this project is to investigate the causal and functional aspects of altered body mass and energy regulation during chronic antidepressant drug treatment.

Methods

Some of the equipment used in these studies has been described in Z01 MH 02405-02 CP.

a) Tissue collection

Tissue was collected from hamsters treated for over four weeks with clorgyline (2 mg/kg/day) or saline. Hamsters were sacrificed at eight phases of the circadian cycle. Pineal organs were carefully dissected and immediately frozen on dry ice. At the same phases blood was collected. After processing, serum samples were assayed in duplicate for ACTH, cortisol and corticosterone using a standard extraction, chromatography and RIA techniques. Pineals were assayed for melatonin content using a RIA technique. Two factor ANOVA was used to analyze data.

b) Autonomic and Behavioral Thermoregulation

Hamsters were chronically treated with clorgyline or saline as described above. Autonomic responses included metabolic rate, evaporative water loss, thermal conductance and core temperature measured at ambient temperatures ranging from 5-35°C. All testing was conducted during the light phase. Behavioral thermoregulation was tested using a temperature gradient. The thermal gradient consisted of a runway (13 cm X 183 cm) that ranged from 15°C at one end to 35°C at the opposite end. Hamsters self-selected preferred temperature on the gradient over the course of twenty-four hours. Position and preferred temperature on the gradient were continuously recorded using infrared detectors and a data acquisition system. The experiments were conducted in collaboration with Dr. Christopher Gordon.

Findings to date

Chronic treatment with the antidepressant drugs clorgyline, lithium or fluoxetine decreases hypothalamic temperature. Compared with saline-treatment, chronic clorgyline-treatment resulted in significantly less whole body, adrenal, white adipose tissue and brown adipose tissue (BAT) mass. In contrast, chronic clorgyline did not significantly alter testis mass. The observations a) that chronic clorgyline was found to decrease brain temperature as well as decrease the mass of the adrenals and the intrascapular BAT, b) that chronic corticosterone or melatonin administration alters BAT thermogenesis and c) that cortisol levels are reported to be increased by 5HT, which is increased by clorgyline, prompted an examination of clorgyline's effects on daily variation in corticosteroid and melatonin levels.

a) Clorgyline effects on the circadian rhythms of cortisol, corticosterone, ACTH and melatonin.

Chronic clorgyline-treatment significantly elevated levels of cortisol ($p=.016$), corticosterone ($p=.03$), and ACTH ($p=.03$), compared with saline-treatment. Although levels of these hormones began to increase at the same time of day during both clorgyline and saline-treatment, the decrease was delayed for several hours by clorgyline. Thus, the duration of the daily peak of corticosteroid levels was expanded by clorgyline.

Pineal melatonin was also found to be significantly elevated by chronic clorgyline-treatment ($p=.0001$). In addition, there was a significant interaction ($p=.012$) between clorgyline-treatment and time-of-day on melatonin level. Pineal melatonin levels began to increase earlier in the dark phase, and decrease later in the dark phase when compared with saline-treated hamsters. Thus, the duration of the daily peak of melatonin levels was also expanded by clorgyline.

b) Clorgyline effects on autonomic and behavioral thermoregulation

Ambient temperature (T_a) significantly affected all autonomic parameters examined in both clorgyline and saline-treated hamsters. Minimum and maximum metabolic rates were observed at

Ta's of 30 and 5°C respectively. Compared with saline-treated hamsters, clorgyline-treated hamsters exhibited an elevated metabolic rate at Ta=5°C ($p=.012$), and tended to increase motor activity at this temperature ($p=.078$). As previously observed, clorgyline significantly decreased body weight ($p=.04$).

When placed on a temperature gradient, saline treated hamsters exhibited a significant circadian rhythm in thermal preference. Hamsters preferred warm temperatures during the light (rest) phase and cooler temperatures during the dark (active) phase. This circadian rhythm in thermal preference was abolished by chronic treatment with clorgyline. Also, compared with saline-treated hamsters, clorgyline-treated hamsters exhibited a marked increase in the level of activity when on the gradient. Overall the distribution of preferred temperature was altered by clorgyline: drug-treated hamsters spent a significantly greater portion of time at temperatures that were either above 32°C, which is the upper limit of thermoneutrality ($p<.05$) or below 28°C, which is the lower limit of thermoneutrality ($p<.05$) in untreated Syrian hamsters. The observation that between 15-30°C, the autonomic responses of clorgyline and saline-treated hamsters were not different, but that their behavioral thermal preference was different, suggests that in Syrian hamsters, clorgyline's predominant effect may be on behavioral thermoregulation.

Significance to Biomedical Research

Chronic antidepressant drug treatment of Syrian hamsters decreases brain temperature and body mass. Oxygen consumption is increased with decreasing ambient temperature. The observations that chronic corticosteroid-treatment diminishes thermogenesis by BAT, and that chronic clorgyline-treatment increases corticosteroid levels, suggest that drug-induced effects on hormones, particularly corticosteroids, could be related to altered thermoregulatory function in Syrian hamsters. Use of behavioral thermoregulatory effectors may be increased to compensate for the diminished capacity of physiological or autonomic effectors, particularly at low environmental temperatures. These studies may provide important new information about the effects of antidepressant drugs on thermoregulation and provide clues that may lead to novel treatments for affective illness.

Proposed Course

Chronic clorgyline treatment of Syrian hamsters elevates plasma corticosterone, cortisol and ACTH and these hormones may have important effects on thermoregulation. We have found (see Z01 MH 02294-08 CP) that this drug also chronically elevates 5HT in the paraventricular nucleus of the hypothalamus, a nucleus containing CRF cell bodies. If corticosteroids do mediate some of the thermoregulatory effects of chronic antidepressant drug treatment in hamsters, then it may be possible to examine the effects of these hormones on brain temperature or thermal preference during chronic corticosteroid or CRF antibody administration. In order to understand effects of drugs on corticosteroid regulation, it will be important to further examine the regulation of ACTH and corticosteroid release by 5HT, as well as by selective 5HT agonists. In addition, to more fully understand clorgyline's effects on corticosteroid regulation, we will measure CRF levels in PVN during clorgyline treatment.

Publications

Duncan, W.C., Gao, B., Wehr, T.A. (1991) Light and antidepressant drugs: interactions with vigilance state, body temperature and oxygen consumption in Syrian hamsters. Sleep 90 J. Horne (Ed), Pontenagel Press, Bochum, pp. 356-359.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02424-05 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) Biological Mechanisms of the Antidepressant Effects of Sleep Deprivation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	T. A. Wehr	Chief, Clinical Psychobiology Branch CPB/NIMH
Others:	N. E. Rosenthal	Chief, Section on Environmental Psychiatry CPB/NIMH
	D. Oren	Clinical Associate CPB/NIMH
	D. Moul	Clinical Associate CPB/NIMH
COOPERATING UNITS (If any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Total sleep deprivation for one night induces temporary remissions in sixty percent of patients with major <u>depression</u>; it can also induce mania in bipolar patients. Furthermore, as little as one hour of sleep can trigger depression in patients who have improved after sleep deprivation. Thus, <u>sleep</u> appears to have a depressant effect and wakefulness a mood-elevating effect. These observations have practical implications for the management of affective illness. For example, some patients' depressions can be treated with sleep deprivation, and sleep disruption is sometimes an identifiable and preventable cause of mania. The purpose of this project is to attempt to identify biology mechanisms of the antidepressant effects of sleep deprivation. Identification of biological mechanisms would increase understanding of the pathogenesis of depression and mania and could be expected to lead to new, rapidly acting drug treatments for depression and mania.</p> <p>The current project is designed to test a hypothesis that thermoregulatory mechanisms underlie the mood-altering effects of sleep and sleep deprivation. The hypothesis is based on our observation that many of the physiological responses to sleep resemble responses to <u>heat exposure</u>. Like heat exposure, sleep onset stimulates secretion of sweat, <u>prolactin</u> (PRL) and <u>growth hormone</u> (GH), and it inhibits metabolic heat production and secretion of <u>thyrotropin</u> (TSH and <u>triiodothyronine</u> (T3). Like <u>cold exposure</u>, sleep deprivation has opposite effects. We are testing the hypothesis that the heat-like property of sleep and the cold-like property of sleep deprivation are responsible for their clinical and neuroendocrine effects in depressed patients. According to this hypothesis, a warm environment, compare with a cool environment, should blunt the antidepressant and neuroendocrine effects of sleep deprivation. Patients are sleep-deprived on one occasion in an ambient temperature of 33° C, and on another occasion in an ambient temperature of 18° C. Twelve patients have been studied, and the results are consistent with the hypothesis. After sleep deprivation in the cool condition compared with the warm condition antidepressant effects were greater ($p < 0.03$), augmentation of TSH secretion was greater ($p < 0.05$), augmentation of T3 was greater ($p < 0.01$), and suppression of PRL was greater ($p < 0.03$). The results show that the depressant and neuroendocrine effects of sleep can be mimicked by heat exposure.</p> <p>Animal studies being carried out in our laboratory could be interpreted as providing further evidence that cooling is antidepressant (ZO1 MH 02294-06 CP). In hamsters, chronic treatment with three classes of antidepressant drugs (a serotonin reuptake inhibitor, a monoamine oxidase inhibitor, and lithium) lower brain temperature 0.5° C during sleep. In contrast, the antimanic agent, haloperidol, raises brain temperature during sleep. Finally, in patients with winter depression, phototherapy lowers rectal temperature 0.5° C during sleep.</p>		

Project Description:

Total sleep deprivation for one night induces temporary remissions in sixty percent of patients with major depression. Like other antidepressant agents, sleep deprivation can also induce mania in bipolar patients. Furthermore, sleep, as brief as one hour in duration, can re-induce depression in patients who improve after sleep deprivation. These observations have practical implications for the management of affective illness. For example, some patients' depressions can be treated with sleep deprivation. Unfortunately, however, many patients relapse during recovery sleep after they have responded to sleep deprivation. In these cases repeated partial sleep deprivation or coadministration of drugs may help to sustain the antidepressant response to the procedure. In the course of bipolar illness, emotional states and life events which disrupt sleep may cause mania through the sleep deprivation mechanism. In many cases these factors are identifiable and preventable causes of mania. The effects of sleep and sleep deprivation on affective illness are also important clues to the biological mechanisms which cause depression and mania. Stated simply, a process connected with wakefulness is capable of improving depression and causing mania, and a process connected with sleep is capable of improving mania and causing depression (at least in patients who relapse during recovery sleep). Clearly, if we had more knowledge of these biological mechanisms, we would understand more about the causes of depression and mania. Knowledge of biological mechanisms of sleep deprivation would undoubtedly suggest radically new types of pharmacological treatments for depression and mania. Drugs which mimic or block the effects of sleep deprivation might be used to treat depression and mania, respectively, and, like sleep deprivation, they might be expected to act much more rapidly than currently available drugs. The purpose of this project is to identify biological mechanisms of the antidepressant effects of sleep deprivation by investigating the effects of sleep deprivation on biological variables, such as hormones and neurotransmitter metabolites, which might mediate its antidepressant effects. In assessing the results of our, and others' earlier work in this area it became apparent that many of the physiological effects of sleep are essentially the same as effects of heat exposure. This similarity exists because, at the level of hypothalamic temperature control mechanisms, perturbations caused by sleep onset and heat exposure are fundamentally the same. During heat exposure hypothalamic temperature rises to a level higher than the set-point for temperature regulation, generating a positive error signal in the controller. After sleep onset the set-point of the controller is reset 0.5°C lower than during wakefulness. Since hypothalamic temperature does not immediately drop 0.5°C , because of the thermal inertia of the body, the resetting of the set-point generates a positive error signal in the controller, just as occurs with heat exposure. We hypothesized that, if this heat-like property of sleep were responsible not only for its neuroendocrine effects but also for its depressant effects, that it should be possible to block or blunt the antidepressant effects of sleep deprivation by carrying out the procedure in a warm environment (in effect, substituting heat for sleep). We tested this hypothesis by comparing the antidepressant effects of sleep deprivation in warm and cool environments.

Methods:

Patients were sleep deprived for one night on two different occasions. On one occasion they were sleep deprived in a very warm environment (33°C), on another occasion they were sleep deprived in a very cool environment (18°C). During a 24 hour period with their usual sleep, and during the two 24 hour periods of sleep deprivation in warm and cool environments, clinical state, body temperature, and hormone secretion were assessed. Before and after sleep deprivation, raters who were blind to the sleep deprivation conditions evaluated mood with the Hamilton Rating Scale for Depression. Patients also evaluated themselves with analogue mood self-rating scales, the Stanford Sleepiness Scale (SSS), and the Profile of Mood States (POMS). For 24 hours in each of the three conditions, blood samples were drawn every hour and subsequently analyzed for plasma levels of TSH, PRL, Free T3, Free T4, melatonin and cortisol.

Findings to Date:

Twelve depressed patients completed the study. In these subjects, rectal temperature and PRL levels were higher, and TSH and FT3 levels were lower, during sleep deprivation in the warm environment compared with the cool environment. Antidepressant effects of sleep deprivation were greater in the cool condition than in the warm condition. All of these results are consistent with predictions based on our hypothesis and support the idea that thermoregulatory adjustments after sleep onset are partly responsible for the depressant effects of sleep.

Significance to Biomedical Research:

Our research suggests that thermoregulatory mechanisms may play an important role in the pathophysiology of depression and mania, and in the antidepressant effects of sleep deprivation. This finding is consistent with the results of animal studies of antidepressant drug mechanisms that are currently in progress in our laboratory (ZO1 MH 02294-06 CP). These drug studies could be interpreted as providing further evidence that cooling is antidepressant. In hamsters, chronic treatment with three classes of antidepressant drugs (a serotonin reuptake inhibitor, a monoamine oxidase inhibitor, and lithium) lowers brain temperature 0.5° C during sleep. In contrast, the antimanic agent, haloperidol, raises brain temperature during sleep. Finally, in patients with winter depression, phototherapy lowers rectal temperature 0.5° C during sleep.

Thus, processes associated with brain-cooling may be important in the antidepressant mechanisms of sleep deprivation therapy, antidepressant drugs, and phototherapy.

Proposed Course:

Several additional patients will be studied before the project is complete.

Publications:

Wehr TA: Manipulations of sleep and phototherapy: Non-pharmacological alternatives in the treatment of depression. *Clinical Neuropsychopharmacology* 1990; 13[Suppl 1]:S54-S65.

Wehr TA: Sleep-loss as a mediator of diverse causes of mania: A longitudinal single-case study. *British Journal of Psychiatry* 1991; 159:576-578.

Wehr TA: Improvement of depression and triggering of mania by sleep deprivation. *Journal of the American Medical Association* 1992; 267:548-551.

Wehr TA: A brain-warming function for REM sleep. *Neuroscience and Biobehavioral Reviews*. 1992; in press.

Wehr TA: The mood-elevating effects of sleep deprivation . In reply. *Journal of the American Medical Association* 1992; 267:2605.

Kasper S, Wehr TA: The role of sleep and wakefulness in the genesis of depression and mania. *L'Encephale* 1992; 18:45-50.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02426-05 CP																												
PERIOD COVERED October 1, 1991 to September 30, 1992																														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Physiology of sleep and sleep loss																														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 30%;">C.A. Everson</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 30%;">CPB/NIMH</td> </tr> <tr> <td colspan="4" style="padding-top: 10px;">Others:</td> </tr> <tr> <td></td> <td>T.A. Wehr</td> <td>Chief</td> <td>CPB/NIMH</td> </tr> <tr> <td></td> <td>L. Sokoloff</td> <td>Chief</td> <td>LCM/NIMH</td> </tr> <tr> <td></td> <td>C. Smith</td> <td>Research Chemist</td> <td>LCM/NIMH</td> </tr> <tr> <td></td> <td>S. Jerrels</td> <td>Psychologist</td> <td>CPB/NIMH</td> </tr> <tr> <td></td> <td>M. Jackson</td> <td>Biologist</td> <td>CPB/NIMH</td> </tr> </table>			PI:	C.A. Everson	Senior Staff Fellow	CPB/NIMH	Others:					T.A. Wehr	Chief	CPB/NIMH		L. Sokoloff	Chief	LCM/NIMH		C. Smith	Research Chemist	LCM/NIMH		S. Jerrels	Psychologist	CPB/NIMH		M. Jackson	Biologist	CPB/NIMH
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	S. Jerrels	Psychologist	CPB/NIMH																											
	M. Jackson	Biologist	CPB/NIMH																											
COOPERATING UNITS (if any) H.L. Reed, LTC, Dept. of Environmental Medicine, Naval Medical Research Inst., Bethesda, MD; C. Mudd, Biomedical Engineering and Instrumentation, NCRR, NIH																														
LAB/BRANCH Clinical Psychobiology Branch																														
SECTION Subsection on Sleep Studies																														
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892																														
TOTAL STAFF YEARS 3	PROFESSIONAL: 1	OTHER: 2																												
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p><u>Sleep</u> is ubiquitous among mammals, essential for life, and implicated in the pathogenesis of a variety of physical and mental disorders. Studies were carried out to elucidate further its function and the consequences of its deprivation.</p> <p>Prolonged <u>sleep deprivation</u> in rats causes a negative energy balance and malnutrition symptoms, despite normal integrity of intermediary metabolic pathways as well as increased food consumption. Dietary <u>nutrient</u> composition, which exerted a negligible influence under normal conditions, was found to interact strongly with sleep deprivation, affecting the time-course and development of pathologies.</p> <p>The reason for death from sleep loss has long been unresolved despite numerous morphological, histological, clinical chemistry, and hematological analyses. We found that deprivation eventuates in a breakdown of host <u>immune defense</u> and invasion of the bloodstream by opportunistic, toxigenic microbes. This finding not only identified the events predisposing to death, it implicates <u>cytokines</u> in the mediation of pathophysiology and suggests that sleep preserves <u>immunocompetence</u>.</p> <p>The <u>thyroid hormone</u> profile during sleep deprivation is abnormal and unusual. TRH challenge tests resulted in suppressed plasma free T₄, but normal free T₃, and normal basal and stimulated TSH levels (not expected from low T₄). These findings among others indicate a rapid conversion of T₄ to T₃. Also, low plasma T₄ without increased TSH indicates central hypothyroidism, possibly due to either TSH that is not bioactive or TRH that is suppressed secondary to increased <u>brain temperature</u>.</p> <p>The sleep-deprivation-induced increase in whole-body catabolism is not reflected in local rates of glucose utilization in the brain, which tend to be decreased. Brain temperature and <u>brain glucose utilization</u> become dissociated, due to either an in brain usage of alternate substrates or decreased blood flow.</p>																														

Project Description:

Sleep is a vital biological process that is implicated in the maintenance of both physical and mental health. To understand the role of sleep as an etiological or pathogenic agent in abnormal states we must first study sleep directly, both to determine its function and how the effects of sleep deprivation are mediated.

Methods:

Prolonged sleep deprivation in rats was accomplished using a yoked control paradigm that has been shown to be highly selective for the deprivation of sleep, while interfering little with normal waking activities (see Z01MH0242601CP). Indwelling catheters are implanted into the carotid artery and jugular vein for certain procedures, such as the brain metabolism study. (We have been uniquely successful in maintaining catheter patency for daily blood sampling for up to five weeks required for recovery, baseline, and experimental conditions.) For other procedures, brain and body temperature measurements are acquired with the use of thermistors implanted stereotaxically into discrete brain regions and telemetric transmitters placed in the peritoneum. Assays, autoradiography, and microbiological techniques are completed according to established procedures.

Findings to Date:

Prolonged sleep deprivation in the rat causes an unexplained catabolic state, secondary malnutrition symptoms, and mortality. We have determined that the severity of the malnutrition symptoms, as well as survival time, is partially dependent on the nutritional composition of the diet and reflects responses of intermediary metabolism to nutrient and energy availability. Diet composition interacts strongly with sleep deprivation, affecting the time-course and development of pathologies, whereas it exerts a negligible influence on body weight regulation under normal conditions.

The vital function impaired by sleep deprivation that results in death in animals has long been a mystery. Its identification would tell us not only what type of physical harm is preventable with sleep, but it might also point to an essential function that is normally served by sleep. This year, we discovered that sleep deprivation is lethal in rats because host defenses against endogenous pathogens break down, allowing opportunistic, toxigenic organisms into the bloodstream. We suspected that the lethal agent had to be toxigenic for several reasons: (1) there was a lack of systematic morphological and histopathological changes, (2) the moribund state was not accompanied by seizures, convulsions or diarrhea that are indicative of specific organ-system dysfunction, and (3) sleep can prevent the mortality and readily reverse the pathology without evidence of permanent damage, whereas neurologic damage, for example, would not be expected to recover quickly. No fever occurred in spite of the microbe invasion; temperature, which was evaluated every 30-seconds throughout the 24-hours of each day, decreased to hypothermic levels, suggesting impaired inflammatory responses. The most likely explanation for the unexplained hypothermia during a state of increased heat production might be a decrease in vascular resistance, as is found in human bacteremia-related hypothermia. Future directions of this work include investigation of cytokine mediation of the pathophysiology of prolonged wakefulness. Cytokines are known for their potentially highly catabolic and

deleterious effects. Presumably, cytokines would be activated days or weeks before bacteremia became manifested, and therefore, might have mediated sleep-deprivation pathophysiology.

A role of sleep in immunocompetence has been presumed but never proven. Supportive evidence for such a role comes from strong associative relationships between sleep and changes in immune function parameters. Establishing an active role of sleep in immune function has been difficult because sleep might simply be an accompaniment of health rather than an essential restorative process. For example, animals that sleep and recover after microbial challenge might not have been as sick as those that do not sleep much and do not recover. We plan to investigate whether sleep is a necessary part of the acute phase response to infection and whether it is responsible for recovery of impaired immunocompetence that was caused by sleep deprivation. For example, if sleep is the only variable manipulated in an animal that developed bacteremia as a consequence of sleep deprivation, and that animal survives, the first strong causal direction between sleep and restoration of an impaired function might be provided.

In spite of the life-threatening state caused by sleep deprivation, there is an absence of significant findings in clinical chemistry and hematology parameters, except for plasma alkaline phosphatase (ALP). ALP increases early and progressively during sleep deprivation. Changes in ALP are associated with various disease states, such as bone or liver disease. ALP isoenzyme type may provide an important clue to the nature of the early metabolic changes during sustained wakefulness. Our previous analyses suggested that the isoenzyme was not of intestinal origin. In ongoing analyses we are measuring the enzymes osteocalcin and 5'-nucleotidase in previously collected blood specimens to assess whether the ALP increase during sleep deprivation is due to bone turnover or liver enzyme changes. Future inquiries in light of the host defense findings above include an evaluation of whether the ALP increase signifies early macrophage activation (i.e., neutrophilic ALP).

Even short-term sleep deprivation impairs performance in humans, and we assume that subtle organic changes in the brain are responsible for this. However, the effects of sleep deprivation on the neurophysiology of the brain are not well-understood. To determine whether brain metabolism is affected, we have been investigating brain glucose utilization. Based on our initial analyses, brain glucose utilization in sleep-deprived rats is near-normal, but tends to be decreased overall by nearly 10%. Brain temperature, however, is increased, although not to febrile levels, indicating an unusual dissociation between brain metabolism and temperature. These changes are coupled to a dramatic increase in whole-body energy metabolism and eventual development of hypothermia. We are currently completing brain temperature analyses and autoradiography of additional experimental animals from experiments conducted this year. The future direction of this work will be to determine (1) whether the tendency of decreased brain glucose utilization reflects an increase use of alternate substrates (e.g., ketone bodies) by the brain (because body and dietary fat utilization would be expected to be increased during the whole-body hypermetabolism, and would therefore compete with glucose utilization) and (2) whether brain blood flow is decreased. In the latter case, for example, blood might not perfuse and cool the brain normally during sleep deprivation and this might account for the increased temperature of the brain during sleep deprivation. It is these types of changes

that we can measure in animals that can potentially explain pathogenicities in humans, as well.

The thyroid axis is modified by prolonged sleep deprivation. This change in thyroid function might mediate the unexplained and striking increase in whole-body energy expenditure. This year, we ran additional experiments challenging the thyroid axis with thyroid-releasing hormone (TRH) to determine peripheral responses of thyroxine and triiodothyronine (the most metabolically potent form). Nonaugmented basal and stimulated thyroid-stimulating hormone (TSH), and low plasma T_4 , indicate one or more of the following: hypothalamic hypothyroidism, decreased TSH-stimulated thyroïdal release, and increased negative feedback to TSH mechanisms by T_3 , rather than T_4 . Furthermore, low plasma concentration of T_4 would be expected to decrease the predominant peripheral conversion enzyme, type I 5'-deiodinase (5'D-I), and increase the central nervous system conversion enzyme, type II 5'D-II. This could effectively keep the brain in a euthyroid state, and therefore preserve the homeostasis of the central nervous system while the body is undergoing dynamic change. We are planning three experiments to determine whether: (1) the TSH produced by the pituitary is bioactive, and therefore, capable of stimulating thyroid production and release, (2) conversion of T_4 to T_3 is increased (as a potential mediator of catabolism), and (3) there is a tissue specific metabolic response during sleep deprivation; e.g., increased local conversion in the brain. These studies require double-labeling *in vivo* with radioactive tracers of T_4 and T_3 and dual measurement of activity and concentration of these hormones and metabolites by high performance liquid chromatography (HPLC). We are currently working to establish the HPLC technique to measure hormonal concentrations in small, radioactive specimens of blood and tissue.

Significance to Biomedical Research:

Sleep serves a vital function in humans and animals. Furthermore, changes in sleep appear to play an important role in the pathophysiologies of depression and mania. Sleep physiology and sleep function involve many aspects of neurophysiology, the immune system, hormonal systems, and brain and body temperature and metabolism. To understand its clinical implications for higher-order function, we must first study sleep directly, both to determine how its effects are mediated and how physiological changes are induced by sustained wakefulness.

Publications:

Everson CE, Wehr TA. Nutritional and metabolic adaptations to prolonged sleep deprivation in the rat, *Am J Physiol.* In press.

Mudd CP, Everson CA. A 16-channel microcomputer controlled thermistor temperature measurement system for use in physiological studies (35 to 40 °C). In: Temperature, its measurement and control in science and industry. American Institute of Physics, Volume 6. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02430-05 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) The Effects of Antidepressant Drugs on Sensitivity of the Circadian Pacemaker		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Wallace C. Duncan Research Psychologist CPB/NIMH Others: Norio Ozaki Fogarty Fellow, CPB, NIMH Thomas A. Wehr Chief, Clinical Psychobiology Branch		
COOPERATING UNITS (If any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH Bethesda, Maryland 20892		
TOTAL MAN-YEARS: .5	PROFESSIONAL: .25	OTHER: .25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In this project we investigate the physiological properties of the neural pathways that mediate the effect of light on the timing of daily and seasonal biological rhythms. The timing of these rhythms is regulated by a <u>circadian</u> clock located in the <u>suprachiasmatic nucleus</u>. Exposure to light resets the timing of the clock and the pattern of behaviors controlled by the clock. There are two aspects of this research. First, we examine the clock phase-resetting properties of light-pulses of different intensities and durations in drug-free animals. Second, we examine the effects of chronic, antidepressant drug treatments on clock phase-resetting properties of light. There are three major findings of these recent investigations.</p> <p>First, we confirmed that in drug-free hamsters, the photic sensitivity of this pathway is constant throughout the circadian cycle. That is, equivalent quantities of light delivered at different circadian phases produce phase-shifts that are 50% of the maximum response at each phase.</p> <p>Second, these experiments demonstrate that chronic treatment with the antidepressant drug, clorgyline, decreases sensitivity to light, and that the magnitude of this effect is dependent upon the phase of the circadian cycle at which light is delivered. Thus, more light is required to produce a 50% phase-shift response to evening light than to produce a 50% response to morning light. The shift in the daily pattern of sensitivity to light may contribute to the antidepressant properties of this drug. The mechanism of this shift in sensitivity is currently being examined. The drug may alter the integration of light over time.</p> <p>Third, although previously published reports indicate that <u>lithium</u> alters visual responses as measured by electrooculographic and neurophysiological techniques, our recent data suggests that lithium fails to alter the sensitivity or responsiveness of the photic entrainment pathway. These results underscore the view that this photic system, which is responsible for the entrainment of circadian rhythms, is separate from other visual systems. For example, the photic system mediating entrainment has a high response threshold (about 6 log units above the threshold for vision) and integrates light stimuli for up to 45 minutes (in contrast to 1-3 seconds for some rod photoreceptors). The fact that lithium alters visual responses, but not entrainment responses to light underscores the need to conduct further research on this specific and distinct photic system.</p>		

Project Description:

The antidepressant drugs lithium and clorgyline (CLG) increase the period of the circadian pacemaker and phase-delay the onset of wheel-running. In humans, CLG administration is reported to disrupt the activity-wake cycle, and lithium is reported to alter retinal function. These responses may be related to the drugs' effects on the entrainment response to light. Therefore, we are investigating the effects of antidepressant drugs on the sensitivity of the photic pathway which mediates entrainment to the daily light dark cycle. Our goal is to determine more precisely how entrainment responses are affected by antidepressant drugs.

Methods:

1) Experimental equipment

Measurements of wheel-running behavior of Syrian hamsters were collected on a laboratory computer as described in project report Z01 MH 02294-01 CP. We have developed a light chamber that is used to deliver controlled light pulses, and it is described fully in Z01 MH 02430-01 CP.

2) The Effects of Chronic Clorgyline and Chronic Lithium on the Light Sensitivity of the Circadian Pacemaker Entrainment Response.

Group housed Syrian hamsters are treated for two months with clorgyline (2 mg per kg per day) or saline with subcutaneously implanted mini-osmotic pumps. Hamsters are fed a diet of lithium-supplemented chow for several weeks prior to the experiment. Serum lithium levels are measured to document that lithium levels are within the human therapeutic range. During drug treatment, hamsters are transferred to individual containers with running wheels.

Hamsters are maintained in LD 14.5 : 9.5 for about one week and then maintained in continuous darkness for the remaining three weeks of the study except for a single five minute pulse of light, which is administered to hamsters on day eight of continuous darkness. The magnitude of the phase-resetting response at each light intensity is determined by calculating the difference between the average time of wheel-running activity onset for the seven days before, and for the fourteen days after the light pulse.

In order to assess circadian variation of the phase-resetting response, light is administered at two circadian phases: 1.5 and 6 hours after activity-onset (evening and morning, respectively). The sensitivity of the phase-shift response is determined as the amount of light required to yield a threshold response, a half-maximum response and a saturation response. Stimulus-response (SR) curves were analyzed using a four parameter logistic equation (Allfit) which estimated maximum phase-shift response (Rmax), slope (r), and the light intensity required to produce a half-maximal response (i_0).

3) The Effects of N-acetylaspartylglutamate (NAAG), a Putative Transmitter of the RHT, on the Phase-Resetting Response

In collaboration with Dr. M.A.A. Namboodiri, Department of Biology, Georgetown University, we are evaluating whether NAAG can induce the circadian phase-resetting response of the circadian pacemaker. Hamsters are implanted with a chronic cannula targeting the SCN region of the hypothalamus. Untreated hamsters follow the protocol described in paragraph 2 above, but receive a 0.5 μ l microinjection of NAAG (0.1-10.0 mM) in place of the light pulse. Placement of the cannula tip is histologically verified following the experiment. The dose response and direction of the photomimetic phase-shift response of NAAG-injected animals is compared with the photic response of the light-pulsed animals. If NAAG is a transmitter that mediates the phase-resetting response, we anticipate using it to investigate effects of drugs on circadian rhythms.

Findings to Date:

1) The Effects of Chronic Clorgyline and Chronic Lithium on the Light Sensitivity of the Circadian Pacemaker Entrainment Response.

Stimulus-response (SR) curves were analyzed using a four parameter logistic equation (Allfit) which estimated maximum phase-shift response (R_{max}), slope (r), and the light intensity required to produce a half-maximal (50%) response (i_0).

Comparison of SR curves measured at CT13.5 and CT18 following saline-treatment indicated these curves shared similar half-saturating light intensities (i_0). These curves were statistically distinguished by the combined parameters R_{max} and r . In contrast, clorgyline-treatment significantly increased i_0 at CT13.5 compared with saline at CT18, as indicated by a significant loss of fit when SR curves were constrained by a common i_0 . Thus, clorgyline decreased the hamster's sensitivity to phase-resetting by evening light when compared to morning light. Clorgyline and saline SR curves measured at CT18 were statistically distinguished by the combined parameters R_{max} and i_0 . Lithium, control, and saline SR curves measured at CT18 shared common R_{max} , r , and i_0 parameters. Experiments evaluating the effects of light pulses administered to lithium-treated hamsters at CT13.5 are currently being analyzed.

2) The Photomimetic Effects of NAAG, a Putative Transmitter of the RHT, on the Entrainment Response

Data analyzed in the past year indicate that NAAG (30mM) administered in the vicinity of the SCN produced small phase delays in the onset of wheel-running activity. The magnitude of the phase delay was variable. Locations of the brain probes were verified by histological techniques. All the cannula were found to be either within the SCN itself, or within 200 μ of the dorsal boundary of the SCN. There was little circadian variation in the direction of the entrainment response using 3 mM doses of NAAG, and this response was not similar to the response to light administered at the same time of day.

Significance to Biomedical Research:

These studies explore the functional and neurophysiological aspects of photic input to the circadian system. This work contributes to our understanding of how antidepressant drugs alter photic input to the circadian system. These experiments suggest that the sensitivity of the photic system mediating entrainment to light-dark cycles does not vary as a function of time-of-day in drug-free hamsters. In contrast, chronic clorgyline-treatment does decrease sensitivity of the entrainment response to evening light compared with morning light. The fact that lithium does not appear to alter the photic sensitivity of the entrainment mechanism is of interest since previous research has demonstrated that this drug alters retinal and/or visual function. These findings underscore the need to conduct further research on the unique features of the photic entrainment system. The fact that clorgyline, but not lithium, alters the entrainment response is significant because this response may be related to the drugs' therapeutic properties.

Proposed Course:

These results clearly indicate that some, but possibly not all antidepressant drugs alter the photic responsiveness of the circadian system (the MAOI, clorgyline does, but our early data suggest that lithium does not). During the next year we will complete the studies on lithium in order to fully determine its effects on the daily variation in photic sensitivity of the circadian pacemaker. Also, studies with the drug clorgyline will be extended to address how temporal integration of photic input is altered by drug treatment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02494-03 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) Regulation of Human Biology By Changes In Daylength (Photoperiod)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	T.A. Wehr	Chief, Clinical Psychobiology Branch CPB/NIMH
Others:	D. Moul	Clinical Associate CPB/NIMH
	G. Barbato	Visiting Fellow CPB/NIMH
COOPERATING UNITS (# any)		
LAB/BRANCH Clinical Psychology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Most living things exhibit striking seasonal changes during the course of the year. In many cases, the changes are known to be induced by seasonal changes in daylength, or photoperiod. Changes in the photoperiod generally affect animals in two ways. First, the constant daily expansion or contraction of the photoperiod can cause an animal's daily <u>activity</u> or rest phase to expand or contract, so that it matches the photoperiod. Second, specific changes in the photoperiod can trigger new behaviors, such as <u>hibernation</u>, migration, or <u>breeding</u>, that represent adaptations to conditions that prevail during a particular season. These new behaviors are often accompanied by and depend on marked changes in the biochemistry, physiology and anatomy of the organism.</p> <p>Basic researchers have proposed that the photoperiod controls the organism through its effects on a <u>biological clock</u> mechanism that employs two separate <u>circadian oscillators</u> to track the constantly changing times of dawn and dusk through the course of the year.</p> <p>This project is designed to investigate whether human biology is similarly subject to control by changes in the photoperiod. We monitor daily patterns of <u>sleep</u>, <u>hormones</u> and <u>body temperature</u> in individuals who live for one month on a schedule of light exposures that simulate a natural winter day. They move about freely in ambient natural and artificial <u>light</u> for ten hours each day but are confined to a dark room for fourteen hours each night. For comparison, we make similar measurements in the same individuals while they live for one week on a conventional schedule in which they are exposed to ambient light for sixteen hours each day and sleep in a dark room for eight hours each night.</p> <p>The results of the experiment suggest that the human circadian system is similar to that of other animals—that it is composed of two subsystems, one of which is synchronized with dawn and the other with dusk. This conclusion is based on the following observations: After night was artificially expanded to fourteen hours, sleep divided into two components that gradually moved apart. The evening component (E) was associated with the daily nadir of <u>cortisol</u> secretion, onset of <u>melatonin</u> secretion, onset of <u>prolactin</u> secretion, onset of sleep, peak of <u>slow wave sleep</u> and decline in body temperature, and it was entrained to dusk. The morning component (M) was associated with the daily peak of cortisol secretion, offset of melatonin secretion, offset of prolactin secretion, peak of <u>REM sleep</u>, rise in body temperature and onset of wakefulness, and it was entrained to dawn. Changes in the photoperiod induced changes in the timing of melatonin, temperature, cortisol and thyrotropin circadian rhythms that were independent of one another. This observations suggest that each of these rhythms may be controlled by separate pairs of slave oscillators. The results are likely to have important implications for human biology and medicine.</p>		

Project Description:

Most living things exhibit striking seasonal changes during the course of the year. In many cases, the changes are known to be induced by seasonal changes in daylength, or photoperiod. Changes in the photoperiod generally affect animals in two ways. First, the constant daily expansion or contraction of the photoperiod can cause an animal's daily activity or rest phase to expand or contract, so that it matches the photoperiod. Second, specific changes in the photoperiod can trigger new behaviors, such as hibernation, migration, or breeding, that represent adaptations to conditions that prevail during a particular season. These new behaviors are often accompanied by and depend on marked changes in the biochemistry, physiology and anatomy of the organism.

Animal researchers have proposed that the photoperiod controls the organism through its effects on a biological clock mechanism that employs two separate circadian oscillators to track the constantly changing times of dawn and dusk through the course of the year. This model fits observations that many animals have bimodal patterns in their daily behavior, and it provides a mechanism that could account for their ability to expand and compress their activity or rest phases to match the photoperiod. Since photoperiodism rules the lives of most plants and animals, it seems likely that it governs human behavior and physiology, too. However, to our knowledge, this possibility has not been experimentally investigated. To address this question, we are monitoring daily patterns of sleep, hormones and body temperature in individuals who live for one month on a schedule of light exposures that simulate a natural winter day.

Methods:

The individuals move about freely in ambient natural and artificial light for ten hours each day but are confined to a dark room for fourteen hours each night. For comparison, we make similar measurements in the same individuals while they live for one week on a conventional schedule in which they are exposed to ambient light for sixteen hours each day and sleep in a dark room for eight hours each night.

The individuals were instructed that while they are in the dark room they are to remain at bedrest and to sleep whenever possible, except when it is necessary to use an adjoining, dark bathroom. No other activities, such as exercise or listening to music, are permitted.

Sleep EEG, which is scored in 30-second epochs according to conventional sleep stage criteria, is recorded throughout each night of the study. Rectal temperature, which is sampled every six minutes, is recorded continuously throughout each day of the study.

At 1 p.m. on the afternoon before the last night of each phase of the study an intravenous catheter is inserted in a forearm vein. Beginning at 5 p.m., blood samples are withdrawn through the catheter and collected in heparin-coated tubes every thirty minutes 1) prior to the sleep/dark period, 2) during the 8- or 14- hour sleep/dark period, and 3) during a 29-hour constant routine that lasts from the end of the sleep/dark period at 8 a.m. until 1 p.m. the following day. To avoid disturbing sleep during the sleep/dark period blood samples are withdrawn through long tubing from a position outside the room. During the constant routine the individuals are kept awake while they sit in very dim light (< 1 lux at eye level) and eat small, 2-hourly, isocaloric meals. The purpose of the constant routine is to minimize or to distribute evenly the effects of factors like sleep, posture, exercise, meals and light, that could alter the levels of hormones and body temperature and thereby distort the intrinsic patterns of their circadian rhythms. Plasma cortisol, prolactin, growth hormone, thyrotropin and melatonin concentrations are measured by radioimmunoassays.

Findings to Date:

The results of the experiment suggest that the human circadian system is similar to that of other animals—that it is composed of two subsystems, one of which is synchronized with dawn and the other with dusk. This conclusion is based on the following observations: After night was artificially expanded to fourteen hours, sleep divided into two components that gradually moved apart. The evening component (E) was associated with the daily nadir of cortisol secretion, onset of melatonin secretion, onset of prolactin secretion, onset of sleep, peak of slow wave sleep and decline in body temperature, and it was entrained to dusk. The morning component (M) was associated with the

daily peak of cortisol secretion, offset of melatonin secretion, offset of prolactin secretion, peak of REM sleep, rise in body temperature and onset of wakefulness, and it was entrained to dawn. When the interval between dusk and dawn was changed, the phase relationship between E and M changed correspondingly. Hence, expansion of the dark phase led to parallel expansions of the rest phase of the activity-rest cycle, the sleep phase of the sleep-wake cycle, the rising phase of the cortisol secretory rhythm, the active phase of the melatonin secretory rhythm, the active phase of the prolactin secretory rhythm, and the hypothermic phase of the body temperature rhythm. In contrast, the change in photoperiod had no discernable effect on the pattern of the thyrotropin circadian rhythm.

The photoperiod experiment revealed interesting homologies between human sleep patterns and behavioral patterns in other animals. Such homologies were readily apparent in the case of the diurnal siberian chipmunk, *Eutamias sibiricus*, for which detailed sleep recordings have been published. In short photoperiods, human beings and siberian chipmunks both exhibit remarkably similar bimodal patterns in their nocturnal sleep profiles. For nocturnal rodents, such as mice, homologies are apparent only when one assumes that the two bouts of nocturnal human *sleep* are governed by the same circadian mechanisms as the two bouts of nocturnal rodent *activity* that are usually seen in recordings of the activity-rest cycle. This assumption implies that the dual-oscillator circadian pacemaker maintains opposite phase relationships to the behavioral rhythms that it drives in the day-active and night-active species, but that it maintains an unvarying phase relationship to the light-dark cycle to which it is entrained. The hypothesis that the pacemaker's phase-relationship to the light-dark cycle is conserved across diurnal and nocturnal species is supported by several other types of observations: 1) The circadian pacemaker's phase responses to light pulses have similar patterns (phase-response curves), with phase-delays in the early subjective night and phase-advances in the late subjective night, in both types of animals. 2) The suprachiasmatic nucleus of the hypothalamus (SCN), which contains a circadian pacemaker, generally exhibits increased electrical multiple-unit activity and decreased glucose utilization in the daytime and decreased multiple-unit activity and increased glucose utilization at night in both types of animals. 3) Certain outputs of the pacemaker, such as the nocturnal melatonin circadian rhythm and the diurnal CSF arginine vasopressin circadian rhythm, maintain phase-relationships to the light-dark cycle that are the same in both types of animals.

The photoperiod experiment provided important new information about the regulation of daily patterns in prolactin secretion. Heretofore, it was believed that the rise in prolactin secretion that occurs each night is triggered by, and depends upon, sleep. However, in short days and long nights, prolactin secretion rose to peak nighttime levels immediately after lights were turned off, even though sleep did not begin until two hours later. Thus, contrary to previous beliefs, sleep is not necessary to trigger the nocturnal rise in prolactin secretion. Preliminary results of further experiments indicate that acutely, darkness, resting and lying down are not sufficient to trigger this rise. Further experiments will be necessary to determine how chronic adherence to the short photoperiod regime dissociates the nightly onset of increased prolactin secretion from the nightly onset of sleep.

So that we could evaluate long-term effects of exposure to short photoperiods (as would occur during the course of the year), we asked two individuals to continue in the short photoperiod regimen for 106 days. Two important preliminary findings emerged from this component of the study. 1) There were no obvious effects of exposure to short days on the individuals' reproductive systems. 2) There was a reversible, twenty percent decrease in size of the individuals' pituitary glands. The functional correlates of this change, if any, have not yet been determined.

The photoperiod experiment unexpectedly served as an instrument to dissect the human circadian system and reveal important information about its structure. We found that the various internal phase relationships between circadian rhythms in melatonin, cortisol, thyrotropin and body temperature changed when the photoperiod was changed, and this result was statistically significant for all possible comparisons between rhythms. The importance of this observation is the implication that the human circadian system is a multi-oscillator system in which circadian rhythms in different variables are controlled by separate slave oscillators. This is a novel finding. No other manipulation of the human circadian system has revealed such dissociations in the phase control of different circadian rhythms.

Significance to Biomedical Research:

This is the first experimental demonstration that human beings have conserved a capacity for their physiology to be regulated by changes in the photoperiod, as occurs in many types of animals. It is also the first demonstration in any animal that the daily patterns of cortisol secretion and rectal temperature are regulated by changes in the photoperiod.

Photoperiodism in animals regulates reproductive function, body weight, metabolism, aggression, motivation and drive, social activity, and many hormonal and neurotransmitter systems. Therefore, the full extent of the impact of photoperiod on human biology needs to be explored further.

An important implication of the results of the photoperiod experiment is that the ambient artificial light to which human beings are ordinarily exposed is sufficiently bright to entrain the dawn- and dusk-tracking circadian oscillators. We base this conclusion on the fact that the two bouts of sleep that drifted apart in the 14-hour dark period, had been compressed into the eight-hour dark period when the individuals were exposed to ordinary ambient artificial light. If this interpretation is correct, then our species' use of artificial light, possibly for 15,000 years or more, has subjected it to an unnatural 16-hour photoperiod that holds it in a perpetual "summer" mode of existence and suppresses and obscures its inherent seasonality. The impact of this unwitting experiment on human behavior, physiology and health remains to be explored more fully.

Changes in the duration of sleep characteristically occur in manic-depressive illness, and these changes are thought to play a role in its pathogenesis, inasmuch as experimental manipulations of the duration of sleep lead to mood changes in patients with the illness. The results of the photoperiod experiments suggest that the photoperiod-tracking system that has been shown to exist in human beings could be responsible for the dramatic changes in sleep duration that occur in manic-depressive illness. Many seasonal changes in animal behavior resemble symptoms of manic-depressive illness. For example, at one time of year, certain animals lose interest in sex and other activities, withdraw from the environment, sleep more, eat more and gain weight. At other times of year opposite changes occur. In light of these analogies, it may be possible that disturbances in the human photoperiodic mechanism lie at the core of manic-depressive illness.

Proposed Course:

Possible future directions include the following:

- 1) The effects of long and short photoperiods on daily patterns in sleep propensity can be assessed in order to determine whether changes in sleep patterns there were induced by exposure to short days resulted from changes in circadian rhythms that gate the occurrence of sleep. Sleep propensity can be measured by recording sleep during short nap-opportunities provided at frequent and regular intervals around the clock.
- 2) Further experiments can be undertaken to investigate the mechanism through which the onset of nightly prolactin secretion becomes dissociated from the onset of nightly sleep in short days and long nights.
- 3) The factors responsible for changes in pituitary size that were observed during exposure to short days and long night can be elucidated. The functional correlates of this change can be investigated.
- 4) To test the hypothesis that photoperiod-responsive mechanisms are involved in the pathophysiology of affective illness, responses of affective patients to photoperiod manipulations can be investigated.

Publications:

Wehr TA: The durations of human melatonin secretion and sleep respond to changes in daylength (photoperiod). *Journal of Clinical Endocrinology and Metabolism* 1991; 73:1276-1280.

Wehr TA, Giesen HA, Schulz PM, Anderson JL, Joseph-Vanderpool JR, Kelly K, Kasper S, Rosenthal NE: Contrasts between symptoms of summer depression and winter depression. *Journal of Affective Disorders* 1991; 23:173-183.

Wehr TA: Response of human sleep and melatonin secretion to changes in photoperiod: Implications for manic-depressive illness, in Racagni G, Brunello N, Fukuda T (eds), *Biological Psychiatry*, Elsevier, Amsterdam, 1991, pp. 783-786.

Wehr TA: In short photoperiods, human sleep is biphasic. *Journal of Sleep Research* 1992; in press.

Wehr TA: Chronobiology, in Kaplan HI, Saddock BJ (eds), *Comprehensive Textbook of Psychiatry*, Baltimore, Williams and Wilkins, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02501-03 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Novel Treatment Modalities for Winter SAD		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	D.A. Oren	Senior Clinical Investigator CPB/NIMH
Others:	N.E. Rosenthal	Chief of Environmental Psychiatry CPB/NIMH
	D.E. Moul	Senior Clinical Investigator CPB/NIMH
	P. Schwartz	Clinical Associate CPB/NIMH
	C. Brown	Program Coordinator CPB/NIMH
	I. Hackett	Nurse CPB/NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology		
SECTION		
INSTITUTE AND LOCATION NIMH Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2	1	1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Projected has been terminated.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH02502-03 CP												
PERIOD COVERED October 1, 1991 to September 30, 1992														
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) An Investigation of Primary Depressives with Secondary Alcoholism														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Ellen Leibenluft</td> <td style="width: 33%;">Medical Officer</td> <td style="width: 33%;">CPB/NIMH</td> </tr> <tr> <td>Others: Pam Madden</td> <td>Psychologist</td> <td>CPB/NIMH</td> </tr> <tr> <td>Norman Rosenthal</td> <td>Chief, Section on</td> <td>CPB/NIMH</td> </tr> <tr> <td></td> <td>Environmental Psychiatry</td> <td></td> </tr> </table>			PI: Ellen Leibenluft	Medical Officer	CPB/NIMH	Others: Pam Madden	Psychologist	CPB/NIMH	Norman Rosenthal	Chief, Section on	CPB/NIMH		Environmental Psychiatry	
PI: Ellen Leibenluft	Medical Officer	CPB/NIMH												
Others: Pam Madden	Psychologist	CPB/NIMH												
Norman Rosenthal	Chief, Section on	CPB/NIMH												
	Environmental Psychiatry													
COOPERATING UNITS (if any)														
LAB/BRANCH Clinical Psychobiology Branch														
SECTION														
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892														
TOTAL MAN-YEARS: .10	PROFESSIONAL: .05	OTHER: .05												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>This project has been completed and terminated.</p> <p>The <u>comorbidity</u> of alcohol dependence with major psychiatric illness, including affective disorders, is receiving increased attention in the psychiatric literature. However, little systematic research has been done on patients with <u>primary depression</u> and <u>secondary alcoholism</u>. These patients, who typically claim to "self-medicate" their depression with alcohol, are interesting to study because their choice of psychoactive substance may provide a clue to the neurochemistry underlying their symptoms.</p> <p>During this period, we completed a pilot study comparing patients with a history of comorbid primary depression and secondary alcohol dependence to two comparison groups: patients with a history of depression but no history of alcohol abuse or dependence, and patients with a history of alcohol dependence but no affective illness. Patients were matched according to age, sex, and Global Assessment of Functioning score. Eleven patients in each group completed a standardized work-up in which we characterized symptoms, family history, and pattern of drug and alcohol abuse. Results indicate that comorbid patients are significantly more likely than depressed patients to meet DSM-III-R criteria for <u>panic disorder</u> ($p=0.04$). Compared to depressed patients, comorbid patients have significantly higher <u>hypomania</u> scores ($p=0.03$) although (with the exception of one comorbid patient) neither the depressed nor the comorbid patients met criteria for bipolar illness. These results indicate that comorbid patients with primary depression and secondary alcoholism may tend to be "trimorbid", with symptoms of depression, anxiety, and alcoholism.</p> <p>In conjunction with the above project, we developed a questionnaire to study patients' use of <u>alcohol</u>, <u>carbohydrates</u>, and <u>caffeine</u> in response to specific depressive symptoms. Test-retest reliability was established. The questionnaire has been administered to normal volunteers and to patients in four separate diagnostic categories: seasonal affective disorder, major depressive disorder, primary depression with secondary alcohol dependence, and alcohol dependence without affective disorder. Patients in the latter two groups did not differ in the reported use and effect of alcohol, so that alcoholics without a history of depression were as likely to report drinking in response to depressive symptoms as were those who had had episodes of primary depression. In terms of caffeine and carbohydrate use, the responses of the patient groups did not differ from each other, but all differed significantly from the normal volunteers. Discriminant function analysis distinguished alcoholics from non-alcoholics in the relationship between the alcoholics' reported drinking, anger, and anhedonia.</p>														

Project Description and Methods:

1. Comparison of Primary Depressives with Secondary Alcoholism, Non-Alcoholic Depressives, and Alcoholics Without a History of Depression

This study was designed to compare systematically: (1) depressed patients who "self-medicate" their depression with alcohol, (2) depressed patients who do not drink, and (3) alcoholics without a history of depression. Patients in the three groups were matched by age, sex, and Global Assessment of Functioning (GAF) score. Each patient received a standardized work-up including the Structured Clinical Interview for DSM-III-R (SCID-R), Structured Interview for DSM-III Personality Disorders, Relative Psychiatric History Questionnaire, Drug and Alcohol Use Inventory (designed to classify alcoholics according to Cloninger's typology), Hamilton Anxiety Scale, Hamilton Depression Rating Scale with addenda for atypical symptoms and for hypomania, three personality inventories, and the Profile of Mood States questionnaire.

Statistical analyses were based on between-group comparisons of the comorbid, depressed, and alcoholic groups. Data were analyzed by means of chi-square tests for categorical variables, and t-tests or analyses of variance with post-hoc t-tests for continuous variables. All tests were two-tailed, and significance was set at $p < 0.05$. Given the hypothesis-generating nature of the study, no corrections were made for multiple comparisons.

2. Development of Self-Medication Questionnaire

The purpose of the questionnaire was to define more precisely the relationship between patients' depressive symptoms and their intake of alcohol, carbohydrates, and caffeine. Previous studies of the question of self-medication have examined patients' reported substance abuse in relationship to global measures of depression. However, it is quite possible that it is particular depressive symptoms, not depression per se, that motivate patients to ingest drugs. Thus, in contrast to questionnaires used previously, this instrument was designed to ask patients about their substance use in response to specific symptoms. In addition, patients were asked their assessment of the effect of the substances on these symptoms.

Test-retest reliability was established using Cohen's kappa. ANOVA and stepwise multivariate discriminant function analyses were used to determine if diagnostic groups differed in the reported use and effect of each of the three substances.

Findings to Date:

1. Comparison of Primary Depressives with Secondary Alcoholism, Non-Alcoholic Depressives, and Alcoholics Without a History of Depression

Thirty-three patients completed the protocol, eleven (6 female, 5 male) in each group. The mean age of the subjects was 46 ± 9 years and the mean GAF score was 74 ± 9 . Ten of the comorbid patients, and all of the patients in the depressed group, met lifetime criteria for major depressive disorder, depressed. One comorbid patient met lifetime criteria for bipolar II disorder.

There were significant differences between the groups in the number of patients meeting DSM-III-R criteria for sedative dependence, and for panic disorder. On post-hoc

comparison, the comorbid group was significantly more likely than the depressed group to meet criteria for panic disorder ($\chi^2=4.14$, $df=1$, $p<0.05$).

The groups differed in the number of relatives reported to have a history of depression, alcoholism, drug abuse, and psychiatric hospitalizations. Post-hoc comparisons showed that the comorbid patients reported a higher percentage of first-degree relatives with a history of drug abuse than the alcoholic group ($\chi^2=7.41$, $df=1$, $p<0.05$) or the depressed group ($\chi^2=7.19$, $df=1$, $p<0.05$).

The Drug and Alcohol Use Inventory revealed no significant differences between the comorbid and alcoholic groups in the pattern and history of alcohol use. Specifically, the age of onset of heavy drinking was similar between the two groups (comorbid= 24.2 ± 8.7 years; alcoholic= 23.5 ± 12.2 years).

On the Hamilton Anxiety scale, there were significant differences between the group means, and post-hoc comparisons revealed that the comorbid group had a significantly higher mean score than the alcoholic group ($F=4.16$, $df=2,30$, $p<0.05$). While there were overall differences between the three groups on mean HDRS scores, there were no significant post-hoc differences between the comorbid and depressed groups.

On the hypomania/mania scale, there was an overall difference in mean scores, and post-hoc comparison showed a significant difference between the comorbid and depressed groups ($F=3.78$, $df=2,30$, $p<0.05$). These differences remained significant ($F=4.36$, $df=2,29$, $p<0.05$) when the comorbid patient with a history of bipolar II disorder was eliminated from the sample. An ANOVA of the item scores on the hypomania scale revealed a significant difference in the symptom pattern of the groups ($F=3.80$, $df=2,30$, $p<0.05$), with comorbid patients having significantly higher irritability scores, and alcoholic patients having significantly higher self-esteem.

2. Development of Self-Medication Questionnaire

The following groups of subjects completed the questionnaire: patients with alcohol dependence (AD) ($N=16$), patients with seasonal affective disorder (SAD) ($N=117$), patients with major depressive disorder (MDD) ($N=35$), patients with comorbid primary depression and secondary alcohol dependence (COM) ($N=24$), and normals (NV) ($N=26$). In addition, 87 SAD patients and 20 NV's completed the questionnaire twice, at a two-week interval, to establish test-retest reliability.

The AD and COM groups had indistinguishable responses concerning the reported use and effect of alcohol. In terms of caffeine and carbohydrate use, the responses of the patient groups did not differ from each other, but all differed significantly from the NV's. Discriminant function analysis distinguished alcoholics from non-alcoholics in the relationship between the alcoholics' reported drinking, anger, and anhedonia.

Significance to Biomedical Research and to the Program of the Institute

The comorbidity of major psychiatric illness and substance abuse is now receiving increased attention. However, patients with primary depression and secondary alcohol dependence have not been studied in a systematic way. The results of this preliminary study indicate that, compared to depressed patients who do not abuse substances, comorbid patients are more likely to meet lifetime criteria for anxiety disorders. In essence, these comorbid patients are "trimorbid", with anxiety, depression, and alcohol dependence.

Furthermore, while the comorbid patients (with one exception) do not meet criteria for bipolar disorder, they tend to have higher hypomania scores than the depressed patients. Compared to depressed patients, comorbid patients may have a heightened state of arousal, causing irritability and anxiety. Comorbid patients may attempt to dampen this arousal through the use of sedative substances, including alcohol.

Results from the self-medication questionnaire indicated that the reported relationship between symptoms and substance use varied depending on the substance in question. Alcoholics without a history of depression were as likely to report drinking in response to depressive symptoms as were those who had had episodes of primary depression. Patients of all diagnostic groups were more likely than normal volunteers to report using caffeine and carbohydrates in response to depressive symptoms.

Obviously, clinicians can target treatment more effectively if the symptoms motivating alcohol use by depressed patients are clearly defined. This work may also help identify depressed patients at risk for alcoholism. Furthermore, these results support the hypothesis that the specific nature of a patient's psychopathology may predispose him to use, or even to abuse, a psychoactive substance. The pharmacology of the abused substance may then provide a clue to the neurochemistry underlying the patient's symptoms.

Proposed Course

These studies have been terminated because they have been completed.

Publications

Leibenluft E, Fiero PL, Bartko JJ, Moul DE, Rosenthal NE: The relationship between depressive symptoms and the self-reported use of alcohol, caffeine, and carbohydrates in four psychiatric patients groups and normal controls. *Am J Psychiatry* (in press)

Leibenluft E, Madden PA, Dick SE, Rosenthal NE: Primary depressives with secondary alcoholism compared to alcoholics and depressives. Comprehensive Psychiatry (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH02503-03 CP																		
PERIOD COVERED October 1, 1991 to September 30, 1992																				
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) A Controlled Study of the Antidepressant Efficacy of Sleep Deprivation																				
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Ellen Leibenluft</td> <td style="width: 33%;">Medical Officer</td> <td style="width: 33%;">CPB/NIMH</td> </tr> <tr> <td>Others: Pam Madden</td> <td>Psychologist</td> <td>CPB/NIMH</td> </tr> <tr> <td>Bridgid Noonan</td> <td>Psychologist</td> <td>CPB/NIMH</td> </tr> <tr> <td>Paul Schwartz</td> <td>Medical Staff Fellow</td> <td>CPB/NIMH</td> </tr> <tr> <td>Thomas Wehr</td> <td>Chief, Clinical</td> <td>CPB/NIMH</td> </tr> <tr> <td colspan="3" style="text-align: center;">Psychobiology Branch</td> </tr> </table>			PI: Ellen Leibenluft	Medical Officer	CPB/NIMH	Others: Pam Madden	Psychologist	CPB/NIMH	Bridgid Noonan	Psychologist	CPB/NIMH	Paul Schwartz	Medical Staff Fellow	CPB/NIMH	Thomas Wehr	Chief, Clinical	CPB/NIMH	Psychobiology Branch		
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SECTION																				
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892																				
TOTAL MAN-YEARS: .20	PROFESSIONAL: .10	OTHER: .10																		
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews																				
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>This project has been completed and terminated.</p> <p>While approximately 60% of depressed patients experience an antidepressant response after <u>sleep deprivation</u> (SD), the clinical utility of this intervention has been limited by the fact that most patients relapse after a night of recovery sleep. The purpose of these studies was to test two possible <u>clinical applications</u> of SD: (1) its ability to hasten the onset of action of <u>antidepressant medication</u>, and (2) its ability to <u>potentiate</u> the action of antidepressant medication in partially-responsive patients.</p> <p>Because patients cannot be blind to the fact that they are being sleep-deprived, it is difficult to design an adequate control condition for SD. The literature indicates that SD in the second half of the night (late SD, or LSD) is a more effective treatment than SD in the first half of the night (early SD, or ESD). Thus, these experiments also tested the utility of using ESD as a control condition for LSD. Patients in both protocols were randomly assigned to ESD or LSD, and followed that schedule of SD for two nights in a row during each of two successive weeks. There was extensive, systematic monitoring of mood and behavior prior to the SD, and for three weeks after the last SD.</p> <p>In the first protocol, patients were drug-free at the beginning of the study, and were started on fluoxetine 20mg qd four days before their first night of SD. Twenty-four patients completed the protocol, eleven in the LSD condition and thirteen in the ESD condition. There are no significant differences between the ESD group and the LSD group in the course of their response to fluoxetine. Thus, while patients improved significantly over the course of the study, it was impossible to distinguish the relative contributions of fluoxetine, SD, and patient expectations to this clinical change. At this point there is no indication that SD can be used to hasten the onset of action of fluoxetine.</p> <p>All patients accepted in the second protocol had been on a stable regimen of antidepressant medication for at least eight weeks, and they were continued on this regimen throughout the study. Twenty-six patients completed this protocol, fourteen in the ESD condition and twelve in the LSD condition. There were no significant differences in the response of the two groups, so ESD does not appear to be an adequate control condition for LSD in this population. However, the SD treatment did appear to decrease significantly the subjects' Hamilton Depression Rating Scale scores. This effect remained significant even after patients whose scores dropped 30% or more upon entry into the study (so-called "placebo responders") were excluded. Therefore, it appears that SD may potentiate the efficacy of antidepressant medications. Prolactin, cortisol, TSH and fT3 levels drawn at 8:00 a.m. at baseline and on the mornings after SD varied significantly over the course of the study but were not related to clinical response.</p>																				

Project Description and Methods:

1. Using sleep deprivation to hasten the onset of action of fluoxetine

Several studies have attempted to use sleep deprivation (SD) to hasten the onset of action of antidepressant medication. These studies have generally had favorable results. However, the studies have had extremely small sample sizes, and have not consistently used blind raters or control groups.

ESD (early sleep deprivation, or SD in the first half of the night) appears to be a less effective antidepressant than LSD (late sleep deprivation, or SD in the second half of the night). Therefore, our studies used ESD as a control condition for LSD. Our hypothesis was that patients assigned to fluoxetine plus LSD would respond faster than those assigned to fluoxetine plus ESD.

At the time of entry into the study, patients were drug-free; met DSM-III-R criteria for major depression, or bipolar disorder, depressed; and had Hamilton Depression Rating Scale (HDRS) scores greater than 14. During a baseline day, HDRS scores were obtained at 8:00 a.m., 1:00 p.m., and 6:00 p.m. (For the latter two ratings, a modified HDRS was used that omits items concerning sleep, diurnal variation, and weight loss.) Profile of Mood States (POMS) and Beck Depression Inventory (BDI) measures were obtained at 7:00 a.m., 12:00 noon, 5:00 p.m., and 10:00 p.m. Blood was drawn at 8:00 a.m. and 10:00 p.m. for the measurement of TSH, T-3, cortisol, and prolactin levels.

Within the next week, patients were started on fluoxetine 20 mg qAM, which was continued throughout the study. Patients were randomly assigned to ESD or LSD. The clinicians performing the HDRS ratings were blind to the patient's sleep schedule. On the fourth and fifth days of drug treatment (days 5 and 6 of the protocol), patients were sleep deprived for two nights in a row. Exactly one week later (days 12 and 13), the two nights of SD were repeated, using the same sleep schedule as the previous week. On the days of SD, as well as after recovery sleep, mood ratings and blood samples were obtained at the same times as on the baseline day. In addition, throughout the study, patients rated their mood on a 100 mm line each day at 7:00 a.m. and 10:00 p.m.

After the course of SD was completed, patients were seen in clinic once a week for three weeks (at 8:00 a.m. on days 19, 26, and 33). At these visits, a HDRS rating was performed, and a blood sample drawn. In addition, patients completed the POMS and BDI.

Data were analyzed using a two-way analysis of variance with post-hoc Newmann-Keuls t-tests.

2. Using sleep deprivation to potentiate antidepressant medication

A second potential clinical application of SD concerns its ability to potentiate the effect of antidepressant medication in partial responders. At least ten studies in the literature have demonstrated that SD can be useful in this regard. However, our study is the first to test this application of SD using a control condition (ESD vs. LSD) and blind raters.

Patients accepted in the study met DSM-III-R criteria for major depressive disorder, or for bipolar disorder, depressed; and had a HDRS score of at least 8. In addition, they had all been on a stable regimen of antidepressant medication for at least eight weeks. This medication regimen was continued throughout the study.

The structure of this study closely resembles that described in (1) above. Patients were randomly assigned to ESD or LSD. Our hypothesis was that LSD will be more effective than ESD in potentiating the effect of antidepressant medication. Patients who had a baseline HDRS less than 15 were randomized separately from those who had a baseline HDRS greater than or equal to 15. The schedule of SD, ratings, blood samples, and clinic visits was identical to that described above. At the end of the protocol, patients who were still depressed were crossed over to the other sleep schedule.

Data were analyzed using a two-way analysis of variance with post-hoc Newmann-Keuls t-tests.

Major Findings:

1. Using sleep deprivation to hasten the onset of action of fluoxetine

Twenty-four patients completed the protocol, eleven in the LSD condition and thirteen in the ESD condition. Overall, there was no significant difference between the two groups in the course of their response to fluoxetine. When the two groups of patients were pooled, there was an overall improvement in their depression over the course of the study ($F=7.67$, $df=23$, $p=0.0000$). Post-hoc Newmann-Keuls tests showed a significant decrease in HDRS scores between the baseline day and day 5 ($p<0.05$). On day 5, the patients had received four days of fluoxetine treatment but have not yet been sleep-deprived. Patients were sleep-deprived on days 5 and 6, and there was another significant drop in depression between day 5 and day 12 ($p<0.05$). Patients were again sleep-deprived on days 12 and 13, but their HDRS scores did not change significantly. The patients did not show significant changes between each of the three follow-up clinic visits (days 19, 26, and 33), although at each of these time points they remained significantly improved over their initial baseline rating.

In sum, while patients improved significantly over the course of the study, it was impossible to distinguish the relative contributions of fluoxetine, SD, and patient expectations to this clinical change.

2. Using sleep deprivation to potentiate antidepressant medication

Twenty-six patients completed this protocol, fourteen in the LSD condition and twelve in the ESD condition. Again, there was no significant difference in clinical course for the two groups, so the groups were combined for the analyses. Overall, there was a significant decrease in HDRS scores over the course of the study ($F=6.16$, $df=5$, $p<0.0001$) and between the baseline day and day 5 ($p<0.05$). Since this latter decrease occurred after patients were enrolled in the study but before they are sleep-deprived, the improvement in clinical status must be due to patient expectations. In order to eliminate these "spontaneous responders" from the sample, we excluded from the remaining analyses the seven patients whose HDRS score dropped 30% or more between the baseline day and day 5. On average, the remaining patients' HDRS scores decreased significantly after the first course of SD ($p<0.05$), so that the lowest mean HDRS rating occurred at 8:00 a.m. on day 12, the morning prior to the second course of sleep deprivation. From day 12 until the end of the study, the patients experienced a gradual relapse.

For analyses of hormonal data, one patient with missing data, as well as fourteen patients taking exogenous steroids, thyroid hormones, or lithium, were excluded. Eleven patients (9 women, 2 men) remained in the sample, 6 in the ESD group and 5 in the LSD

group. There were no significant differences between the ESD and LSD groups on any of the mean hormonal levels at either 8:00 a.m. or 10:00 p.m. Therefore, the ESD and LSD groups were combined for statistical analyses. At 8:00 a.m., there was a significant main effect for day of study for TSH ($F=7.77$, $df=7$, $p<0.00001$), fT3 ($F=2.96$, $df=7$, $p<0.01$), cortisol ($F=2.36$, $df=7$, $p<0.05$), and prolactin ($F=2.69$, $df=7$, $p<0.05$). At 10:00 p.m., none of the hormones showed a significant main effect for day of study. Analyses of variance revealed no differences between spontaneous improvers, responders, and nonresponders on mean hormonal levels at 8:00 a.m. or 10:00 p.m.

All patients completed a questionnaire concerning their expectations of improvement from the assigned sleep schedule. There was no difference between the spontaneous improvers, nonresponders, and responders on their reported expectations. However, because ESD was not an effective control treatment for LSD, we cannot rule out entirely the possibility that patient expectations contributed to the positive SD response. Nonetheless, a significant SD response remained even after we excluded patients who improved upon enrollment into the study, strengthening the argument that the first course of SD was biologically active in the remaining sample. Since we used more rigorous methodology than previous studies, this work is the strongest evidence available that SD may potentiate the action of antidepressant medication in patients who are treatment-resistant, or who have a partial response to medication.

Significance to Biomedical Research and the Program of the Institute

The antidepressant effect of sleep deprivation has been widely studied, both because of its potential advantages as a clinical treatment and because its rapidity facilitates mechanistic studies. However, the clinical studies of SD have been limited by the lack of an adequate control condition. Our attempt to use ESD as a control condition for LSD was unsuccessful, but this important methodological problem bears further consideration.

While the lack of an adequate control condition precludes definitive conclusions, our results provide evidence that SD may help some patients who have a limited response to antidepressant medication. Further study can elucidate the clinical conditions under which SD is most likely to be an effective treatment. In addition, studies of the mechanism of action of SD, and of the relapse after recovery sleep, can yield important data about the pathophysiology of affective illness.

Proposed Course

These projects have been completed and were terminated.

Publications

Leibenluft E, Wehr TA. Is sleep deprivation useful in the treatment of depression? *Am J Psychiatry* 1992; 159-168.

Leibenluft E, Noonan B, Wehr TA: Diurnal variation: Reliability of measurement and relationship to typical and atypical symptoms of depression. J of Affective Disorders (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH-02577-02-CP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Heritability of Seasonal Change in Mood and Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Pamela A. Madden, M.S.

Psychologist

CPB/NIMH

COOPERATING UNITS (if any)

Andrew C. Heath, D.Phil., Dept of Psychiatry, Washington Univ School of Med, St. Louis, MO
Nicholas G. Martin, Ph.D., Queensland Instit of Med Research, Herston, Queensland, Australia

LAB/BRANCH

Clinical Psychology Branch

SECTION

Environmental Psychiatry

INSTITUTE AND LOCATION

NIMH Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02611-01 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) Biological Findings in Hypnnychthemeral Syndrome		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: D. A. Oren	Senior Clinical Investigator	CPB/NIMH
Others: T. A. Wehr	Chief, Clinical Psychobiology Branch	CPB/NIMH
J. Seidel	Research Assistant	CPB/NIMH
I. Hackett	Nurse	CC/NIH
COOPERATING UNITS (If any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In 1983 we reported the first successful treatment of non-24-hour <u>sleep-wake syndrome</u> (<u>hypnnychthemeral</u> syndrome)—an apparently rare disorder. A second patient with this syndrome subsequently contacted us and we have elected to explore further the pathophysiology that may be responsible for the syndrome. Our goal is to employ a case study analysis of this patient's abnormality as a window into understanding various processes that control human circadian rhythms. We conducted a complete historical and hormonal "work-up" of the patient. The patient wore a light meter with computer-chip memory for a week to assess his level and pattern of ambient light exposure. The light meter was calibrated to measure ambient light with the sensitivity of the human eye. In a four-part set of <u>circadian</u> rhythm procedures the patient was admitted four times for assessment of sleep architecture, and 24 hour profiles of body temperature and various body hormones. Circadian hormone profiles were consistent with those of a "free-running" individual. Expected hour of sleep onset was delayed by two to three hours relative to the normal fall in temperature. We found significant abnormalities in the profiles of the patient's thyroid-related hormones, gonadal hormones, and <u>melatonin</u>. This case study-in-progress offers great potential for furthering our understanding of hormonal regulation of the sleep-wake cycle. We plan to explore the abnormal <u>pineal</u> gland and hypothalamic-pituitary-end organ axes in which we have already found abnormalities through endocrine challenge tests. We plan to try various pharmacological approaches to correct the sleep-wake disorder. </p>		

For the past thirteen years, the work in this Branch has focused upon various aspects of biological rhythms. In 1983 we reported the first successful treatment of non-24-hour sleep-wake syndrome (hypnrychthemeral syndrome)—an apparently rare disorder. This patient has subsequently continued to do well with vitamin B₁₂ treatment. That one case has spurred an entire line of research into the use vitamin B₁₂ as a treatment for hypnrychthemeral syndrome and delayed sleep phase syndrome. A second patient with this syndrome subsequently contacted us and we have elected to explore further the pathophysiology that may be responsible for the syndrome. Our goal is to employ a case study analysis of this patient's abnormality as a window into understanding various processes that control human circadian rhythms.

Project Description and Methods

A complete history and physical examination was obtained from the patient. The exam included routine laboratory testing. The patient wore a light meter with computer-chip memory on his wrist for a continuous week to record his level and pattern of ambient light exposure. The light meter was calibrated to measure ambient light with the sensitivity of the human eye. In a four-part set of circadian rhythm procedures the patient was admitted four times for assessment of sleep architecture, measurement of body temperature, and measurement of various body hormones. In the first and third of the four parts, the patient was studied following a night during which he had slept during "normal" hours. In the second and fourth of the four parts, we studied the patient following a day during which he was asleep during the day. During the first and second parts he was permitted to sleep whenever he wished. During the third and fourth parts he was not permitted to sleep and he was confined to a dark room, as part of a "constant routine" procedure. This procedure attempts to divide normally varying patterns of daily experience evenly over the period of investigation, so that observed temperature and hormonal rhythms will reflect underlying circadian patterns rather than acute effects of normally changing variables such as movement, eating, and ambient lighting. The patient was later admitted to the research unit for three days of monitoring of sleep architecture and collection of urine for analysis of 6-sulfatoxymelatonin.

Findings to Date:

We found no evidence of disordered vitamin B₁₂ regulation; serum vitamin B₁₂, methylmalonic acid, and homocysteine levels were normal. Circadian hormone profiles were consistent with those of a "free-running" individual. There were multiple endocrine and circadian rhythm abnormalities: 1) In the constant routine, expected hour of sleep onset was delayed by two to three hours relative to the normal fall in temperature; 2) TSH levels were abnormal, with no evidence of the expected nocturnal rise during sleep deprivation on a night when the patient would normally have slept, and abnormally high levels as large as 10 mU/L during sleep deprivation during a daytime when the patient would normally have slept; 3) Testosterone, FSH, and LH levels were all below normal levels; 4) There was no detectable plasma melatonin, although there were low urinary levels present of the metabolite 6-sulfatoxymelatonin.

Significance to Biomedical Research and to the Program of the Institute:

This case study-in-progress offers great potential for furthering our understanding of hormonal regulation of the sleep-wake cycle. Although hypnrychthemeral syndrome is a rare syndrome, it is debilitating and prevents otherwise highly capable individuals from leading productive lives. If we prove able to understand the causes of the disorder and develop a treatment for it, our understanding of pathophysiology of circadian disorders may be greatly enhanced.

Proposed Course:

We plan to explore the abnormal pineal gland and hypothalamic-pituitary-end organ axes in which we have already found abnormalities through endocrine challenge tests. We plan to try various pharmacological approaches to correcting the sleep-wake disorder.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02613-01 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders) Light and the Eye in Winter Seasonal Affective Disorder (SAD)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: D. A. Oren	Senior Clinical Investigator	CPB/NIMH
Others: N. E. Rosenthal	Chief, Section on Environmental Psychiatry	CPB/NIMH
D. E. Moul	Senior Clinical Investigator	CPB/NIMH
P. J. Schwartz	Senior Clinical Investigator	CPB/NIMH
N. Ozaki	Visiting Fellow	CPB/NIMH
C. Brown	Program Coordinator	CPB/NIMH
F. Meyers	Nurse	CC/NIH
COOPERATING UNITS (If any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION Section on Environmental Psychiatry		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The role of environmental light exposure and the biological abnormalities responsible for winter SAD are unknown. We have pursued the ophthalmic pathophysiology of SAD along two lines: light exposure to the eye, and the response of the eye to light. To examine whether patients with SAD inhabited environments with different light exposure from people without SAD, we evaluated <u>ambient light</u> exposure using a light monitor that measured environmental light levels as sensed by the human eye in 13 patients and 13 age- and sex-matched controls. We found no difference between the two groups. To consider the possibility that <u>SAD</u> patients have abnormal intraocular pressures and that this variable may be sensitive to light therapy, we examined intraocular pressure in 14 depressed patients and 14 age- and sex-matched normal controls subjects before and after periods of two weeks in which subjects received light therapy. We found no difference between the two groups or between the two conditions of light therapy. To assess seasonal changes in <u>light sensitivity</u>, we evaluated <u>dark adaptation</u> in winter and summer in 11 medication-free patients and 19 controls. We found no difference between the two groups or across seasons. To replicate a prior report of abnormal <u>electrooculogram</u> ratios in SAD, we measured this variable in 16 patients and 16 age-, sex-, and medication-matched controls before and after one week of light therapy. The mean EOG ratios were significantly lower in patients than in controls. There was no effect of light therapy upon the ratios. We plan to continue our investigation of electrooculographic abnormalities by repeating these studies in patients and volunteers during the summer season. We also plan to study whether pharmacological methods of enhancing light sensitivity in patients with SAD might prove beneficial to patients with this syndrome. </p>		

Patients with SAD report that their symptoms are highly reactive to their ambient light levels. Although repeated studies have established that enhanced ambient light from a light box is an effective treatment for SAD, the mechanism of its actions remains unknown, as do the fundamental biological and, perhaps, environmental abnormalities responsible for the syndrome. We have been pursuing the pathophysiology of SAD along two lines: one assessing the light that enters the eye, and another assessing how the eye processes that light.

The initial description of SAD in this Branch noted that the symptoms of this syndrome are particularly noticeable during the months of the year when there is the least amount of ambient light exposure. No differentiation has been explored, however, between the ambient exposure of patients who experienced SAD and people without SAD. To assess if there was an environmental difference in the ambient light exposure of SAD patients and people without SAD, we examined light exposure—calibrated to the sensitivity of the human eye—in SAD patients and normal controls.

Studies of the eye began in this Branch with the demonstration that phototherapy was more effective when delivered to the eye than when delivered to the skin. Our subsequent comparisons of green, red, and blue light for SAD also lent support to the putative role of the eye in SAD, since patients were more responsive to green (which is better absorbed by retinal photoreceptors) than to blue or red. We questioned whether retinal dopaminergic activity might play a role in the pathogenesis of SAD. In our subsequent studies of ocular physiology we have documented the absence of a difference between patients and normal volunteers on a variety of psychophysical and electrophysiological measures of ophthalmic function: sensitivity to dim light on a dark adaptation paradigm, amplitude and latencies of pattern visual evoked potentials and pattern electroretinograms, rates of eye blinking, pupillary size, and color vision. This collection of data based on several paradigms of investigation of a large sample of patients suggests that the particular aspects of ophthalmic function that we examined are not critical to the basis of the pathophysiology of SAD. They also provide no support for the idea that retinal dopaminergic function is central to the pathophysiology of SAD and the beneficial effects of light box therapy.

This past year we continued our investigation of eye pathophysiology by exploring two areas of ophthalmic function that other groups have cited as abnormal in SAD. Stojek and colleagues have reported that low intraocular pressure may be a trait marker for SAD. Since melatonin has been reported to lower eye pressure and bright light suppresses melatonin, it is logical to explore whether light therapy would have the effect of raising intraocular pressure and whether low intraocular pressure would might be a marker of SAD. This also would provide further information about the physiological effects of retinal dopamine in so far as dopamine antagonizes the effects of melatonin in the retina. Lam and colleagues have reported that electrooculogram ratios are somewhat low in SAD patients. This finding is consistent with a retinal dopaminergic or serotonergic abnormality and merits replication. We assessed intraocular pressure and the electrooculogram in patients and normal volunteers before and after light therapy.

Project Description and Methods:

Studies of Ambient Light Exposure in SAD:

To examine whether patients with SAD were exposed to different patterns of ambient light than people without SAD, we evaluated ambient light exposure using a light monitor that measured environmental light levels as sensed by the human eye in 13 patients and 13 age- and sex-matched controls. Each subject wore the monitor while awake for a full week. We calculated total amount of light exposure, average amount of light exposure per minute awake, length of photoperiod, and relative distribution of light exposure across the day for each subject. We analyzed these data with paired t-tests. We also determined the median light exposure across the day for each subject. We compared the median light exposure patterns between patients and controls for group and time effects with an analysis of variance. At the completion of each week of light monitoring, we administered the SIGH-SAD depression interview scale to assess each subject's mood.

Studies of Ophthalmic Function in SAD

To consider the possibility that SAD patients have abnormal intraocular pressures and that this physiological variable may be sensitive to light therapy, we examined intraocular pressure in 14 medication-free depressed patients and 14 age- and sex-matched normal control subjects. Technicians who were blind to subjects' medication or light therapy status read pressures with a Kowa HA-1 hand-held applanation tonometer following local anesthesia of each cornea with a fluorescein and benoxinate eye-drop. Subjects were assessed while sitting after two conditions: 1) An "off-light" condition of two weeks in which subjects avoided bright light by wearing one per cent transmittance goggles outdoors on sunny days; and 2) An "on-light" condition of two weeks in which subjects received light therapy. The pressures were compared between patients and controls with an analysis of variance with on- versus off-lights and left versus right eyes as repeated measures.

Because questions about sensitivity to light in SAD have been at the center of several discussions regarding the pathophysiology of SAD, we also evaluated dark adaptation in winter and summer in 11 medication-free patients and 19 controls by means of a Goldmann/Weekers Adaptometer, made available by the National Eye Institute. We examined subjects using a standard paradigm for measuring sensitivity to dim light following a five minute exposure to a bright light. The paradigm identified the threshold level of detectable luminance at over 30 minutes into the dark adaptation. Logarithmic transformations of the luminances were obtained and were analyzed for group and time effects by means of analysis of variance with repeated measures.

In addition, in an attempt to replicate the prior report of abnormal electrooculograms (EOG's) in SAD and to determine if light therapy changes electrooculogram ratios, we measured this variable in 16 patients and 16 age-, sex-, and medication-matched controls. An examiner who was blind to subjects' treatment status measured their EOG using a standard paradigm with an EPIC-2000 system. The EOG was recorded separately and simultaneously from each eye. The stimulus was provided in a Ganzfeld bowl with built-in red-light-emitting diodes. The average saccade voltage of ten eye movements was then calculated for a period of dim light and for a period of bright light measurement. All subjects were studied in two conditions: "off-light," when subjects were untreated for more than one week; and "on-light," following at least one week of light therapy. The data were analyzed with a repeated measures analysis of variance. Correlations between Hamilton depression rating scores on the day of each study and EOG ratios were obtained using the Pearson correlation coefficient.

Findings to Date:

Studies of Ambient Light Exposure in SAD:

Ambient light exposure did not differ between patients and controls. For each factor studied—total light exposure, average light exposure, length of photoperiod, timing of light exposure—there was no significant difference between patients and controls.

Studies of Ophthalmic Function in SAD

There was no difference in intraocular pressures between patients and controls or before and after light therapy. The dark adaptation studies showed no difference between SAD patients and controls and no effect of season on dark adaptation. The mean EOG ratios were lower in patients than in controls ($F=4.97$, $p<0.04$) although there was a great degree of overlap between ratios of patients and controls. There was no effect of light therapy on the EOG ratios. There was no correlation between changes in the Hamilton ratings and in the EOG ratios of each eye.

Significance to Biomedical Research and to the Program of the Institute:

Our documentation of SAD patients and normal controls being exposed to similar environmental lighting conditions suggests that SAD patients are not exposed to less light than people without the syndrome. SAD is therefore less likely to be an "environmental" disease, than a consequence of

abnormal central nervous system processing of a normal environment. That processing is not reflected in differential sensitivity to dim light in patients versus controls or across the seasons. The lack of change in dark adaptation seen across the seasons represents the largest number of subjects in the literature ever assessed for dark adaptive function and contradicts the one previous report in the literature on a sample of only three subjects. Our failure to find any connection between intraocular pressure and SAD or light therapy adds further weight to our previous work casting doubt on the idea that eye abnormalities are an integral part of SAD. Taken together, this series of studies suggests that the loci for the pathophysiology of SAD will be found in brain structures that are more commonly associated with biological rhythms or with depression in general. Such structures would include the hypothalamus, raphe nuclei, lateral geniculate nuclei, pituitary gland, pineal gland, and limbic system. Abnormalities in SAD such as low prolactin and delayed production of melatonin that have been reported by more than one group of investigators are more likely to be secondary to central brain abnormalities than to ophthalmic pathology. The electrooculogram finding remains to be interpreted further. It may be related to abnormal ophthalmic dopaminergic or serotonergic function, but it also might be an artifact of the examination procedure rather than representing an intrinsic ophthalmic defect.

Proposed Course:

We plan to continue our investigation of electrooculographic abnormalities by repeating these studies in patients and volunteers during the summer season. This would allow us to learn if the EOG can be used as a marker of seasonal change in SAD. Other eye-related investigations of SAD seem less promising at this time. We do plan to study whether pharmacological methods of enhancing light sensitivity in patients with SAD might prove beneficial to patients with the syndrome.

Journal Articles

Oren DA, Jacobsen FM, Wehr TA, Cameron CL, Rosenthal NE. Predictors of response to phototherapy in seasonal affective disorder. *Comp Psychiatry* 1992; 33:111-4.

Chapter in a Book

Oren DA, Rosenthal NE. Environmental light, mood, and seasonal affective disorder. In: Björntorp P, Brodoff BN, eds. *Obesity*. Philadelphia: JB Lippincott, 1992;424-35.

Oren DA, Rosenthal NE. Seasonal affective disorders. In: Paykel ES, ed. *Handbook of Affective Disorders*, 2d Edition. London: Churchill Livingstone (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02614-01 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) Evaluation and Treatment of Rapid-Cycling Bipolar Disorder		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	Ellen Leibenluft	Medical Officer CPB/NIMH
Others:	Paul Schwartz	Medical Staff Fellow CPB/NIMH
	Holly Clark	Social Worker CPB/NIMH
	Fran Meyers	Registered Nurse CPB/NIMH
	Thomas A. Wehr	Chief, Clinical Psychobiology Branch CPB/NIMH
	Norman E. Rosenthal	Chief, Section on Environmental Psychiatry CPB/NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.00	PROFESSIONAL: .75	OTHER: .25
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Patients with <u>rapid cycling bipolar disorder</u> experience at least four episodes of affective illness (<u>depression</u>, <u>hypomania</u>, and/or <u>mania</u>) in a year and are frequently resistant to conventional treatments. Therefore, this psychiatric illness warrants further study because of its significant morbidity. In addition, since rapid cycling patients experience frequent mood shifts that are accompanied by dramatic changes in the sleep-wake cycle, they present a promising opportunity to study the relationships between <u>sleep</u>, activity, and mood disorders. This project studies the <u>circadian rhythms</u> of rapid cycling patients and is the first in a series of planned studies designed to elucidate the etiology of rapid cycling bipolar disorder and to test several experimental treatments. </p> <p> In the current study, rapid cycling patients record their mood and sleep on a daily basis over an extended period of time. In addition, since the majority of rapid cycling patients are premenopausal women, we are particularly interested in the relationship between the menstrual cycle and the affective state of rapid cycling women. Each month, female subjects record their menstrual cycles and monitor their urine for the ovulatory LH surge. All patients, both male and female, intermittently wear temperature and activity monitors on an outpatient basis. </p> <p> Patients are admitted to the hospital for brief, intensive chronobiological evaluations twice in the course of the study: once shortly after they switch into the hypomanic state, and once shortly after they switch into the depressive state. The hospitalizations are each 48 hours in duration and consist of a 20-hour "naturalistic day" and a 28-hour constant routine. The constant routine is designed to minimize the effects of light, sleep, activity, and caloric loading on the patient's circadian rhythms. During the hospitalizations, patients wear rectal temperature probes and activity monitors, and blood is withdrawn every twenty minutes through an indwelling intravenous catheter. By analyzing temperature data and by measuring the secretion of <u>melatonin</u>, cortisol, and other hormones, we will have extensive information about the circadian rhythms of rapid cycling bipolar patients, and about how those rhythms differ in the depressed and hypomanic states. Because the project is in its early stages, results are not available at this time. </p> <p> Other projects on the pathophysiology and treatment of rapid cycling are currently being planned. These include trials of L-thyroxine and bright light in the treatment of rapid cycling, and a protocol using Leuprolide acetate, a GnRH agonist, to explore the role of gonadal steroids in rapid cycling in women. </p>		

Project Description and Methods:

This project is designed to study the circadian rhythms of patients with rapid cycling bipolar disorder in the depressed and hypomanic phases of their illness. Patients must meet DSM-III-R criteria for bipolar disorder and must have experienced at least four episodes of major affective illness (major depressive disorder, hypomania, or mania) within the last year. Throughout the protocol, patients remain on a stable regimen of psychotropic medication prescribed by their physician in the community. They complete daily self-rating forms concerning their mood state and hours of sleep and rest and intermittently wear activity monitors or rectal temperature monitors. Each month, premenopausal women monitor their urine for the ovulatory LH surge and record the onset of menses.

Each patient is admitted to the hospital for brief, intensive chronobiological evaluations twice in the course of the study: once shortly after the switch into the hypomanic state, and once shortly after the switch into the depressive state. These hospitalizations are each 48 hours in duration and consist of a 20-hour "naturalistic day" and a 28-hour constant routine. Throughout the entire hospitalization, blood is withdrawn every 20 minutes through an indwelling intravenous catheter, and patients wear a rectal temperature probe and a light and activity monitor. During the naturalistic day, the patients ambulate around the hospital unit, are exposed to ordinary room light, and sleep ad lib with EEG leads attached. The constant routine, on the other hand, is designed to minimize the distorting or masking effects of sleep, activity, and caloric loading on the patient's circadian rhythms. Therefore, during the constant routine, the patient remains awake in a dimly lit room with limited activity. She is given food and one-twelfth of her daily medication every two hours.

Blood drawn during the naturalistic day will be used to determine levels of prolactin, growth hormone, T3, testosterone, LH, FSH, and sex-hormone binding globulin. In women, levels of estrogen and progesterone will also be measured. Blood drawn during the constant routine will be used to determine levels of TSH, cortisol, and melatonin.

Major Findings

Twelve patients are currently enrolled in the protocol, and five inpatient evaluations have been completed. Since the project is in its early stages, results are not yet available.

Significance to Biomedical Research and the Program of the Institute

Patients with rapid cycling bipolar illness have a particularly severe and disabling psychiatric disorder. They are frequently unresponsive to lithium and other psychotropic medications. This study is designed to elucidate the pathophysiology of rapid cycling and may provide data that can be used to design new treatment interventions. For example, if rapid cycling patients have circadian rhythm abnormalities, they may respond to treatment with bright light. In addition, results from this study may provide information about the mechanisms regulating sleep duration in bipolar patients.

Proposed Course

In addition to completing the study described above, we are currently designing two other protocols on the etiology and treatment of rapid cycling bipolar disorder. The first will use L-thyroxine as an experimental treatment while exploring the effects of thyroid hormone administration on circadian rhythm regulation. The second will use Leuprolide

acetate, a gonadotropin releasing hormone agonist, to explore the role of gonadal steroids in the etiology and treatment of rapid cycling bipolar disorder in women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02615-01 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) The Role of Gonadal Steroids in Regulating Circadian Rhythms in Women		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: Ellen Leibenluft, M.D.	Medical Officer	CPB/NIMH
Others: Peter J. Schmidt, M.D.	Chief, Unit on Reproductive Endocrine Studies	BPB/NIMH
David R. Rubinow, M.D.	Chief, Section on Behavioral Endocrinology	BPB/NIMH
Thomas A. Wehr, M.D.	Chief	CPB/NIMH
COOPERATING UNITS (If any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: .30	PROFESSIONAL: .30	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>There is an extensive body of literature indicating that estrogen and progesterone play important regulatory roles in the <u>circadian rhythms</u> of hamsters and rats. However, this area has received little systematic attention in humans. This protocol is designed to study the circadian rhythms of normal women under pharmacologically controlled hormonal conditions. Normal volunteers are treated with Leuprolide acetate, a gonadotropin releasing hormone (GnRH) agonist, for three months. This treatment reversibly suppresses endogenous secretion of gonadotropins, <u>estrogen</u>, and <u>progesterone</u>. During one of these months, subjects receive estrogen replacement, while they receive progesterone replacement during another month. Thus, subjects can be studied in three pharmacologically controlled hormonal conditions: the <u>hypogonadal</u> state, estrogen alone, and progesterone alone. In each of these conditions, the subjects' circadian rhythms are studied through the use of <u>sleep</u> logs, wrist activity monitors, and rectal temperature monitoring. In addition, during a 48-hour period in each of the conditions, patients are admitted for intensive evaluation that includes frequent blood sampling of prolactin, melatonin, and growth hormone, as well as a constant routine procedure designed to minimize the masking effects of sleep, light, and caloric loading on circadian rhythms. Recruitment of subjects is beginning at this time, so results are not yet available.</p> <p>This line of research is important because of the possible etiologic links between sleep and affective illness, and between gonadal steroids and affective illness. In addition, the protocol may elucidate the effects of hormone replacement therapy on circadian rhythms in normal women.</p>		

Project description and methods:

This project is being conducted in collaboration with the Biological Psychiatry Branch's protocol on the central nervous system effects of pharmacologically induced hypogonadotropic hypogonadism with and without estrogen and progesterone replacement. Normal volunteer women are treated with Leuprolide acetate (Lupron), a gonadotropin releasing hormone agonist, for three months. This treatment suppresses endogenous secretion of LH, FSH, estrogen, and progesterone. For one month of the Lupron treatment, subjects are given replacement estrogen, and for another month they are given replacement progesterone. The purpose of the project is to study the subjects' circadian rhythms in each of these pharmacologically controlled conditions, and thus to elucidate the role of estrogen and progesterone in the regulation of circadian rhythms in humans.

Throughout the study, subjects will record their sleep and rest periods on a standardized form each day. They will wear a wrist activity monitor for one week of each of the three hormonal conditions and spend one night of each condition in the hospital for sleep EEG recording and rectal temperature monitoring. In addition, they will spend a 48-hour period of each condition in the hospital for intensive study. During the first 24 hours of each hospitalization, the subjects will undergo rectal temperature monitoring, EEG sleep recording, and frequent blood sampling via an indwelling intravenous catheter. Subjects will be kept in a dimly lit room and blood will be drawn to measure melatonin, prolactin, and growth hormone. During the second 24 hours of the hospitalization, subjects will remain in the dimly lit room under constant routine conditions, including sleep deprivation, limited activity, and feedings every two hours. The purpose of the constant routine is to obtain unmasked temperature data in each of the hormonal conditions.

Major Findings

Recruitment for this project is beginning at this time.

Significance to Biomedical Research and the Program of the Institute

While there is considerable literature to indicate that gonadal steroids modulate the behavior of circadian pacemakers in female rats and hamsters, there has been little systematic investigation of the effects of gonadal steroids on the human sleep-activity cycle. Given the possible etiologic links between sleep and affective illness, and between gonadal steroids and affective illness, this line of research is an important one to pursue. In addition, the results of this protocol may provide useful information about the effects of hormone replacement therapy on circadian rhythms in postmenopausal women. Since the subjects will be in pharmacologically controlled hormonal states, this protocol provides a unique opportunity to explore the influence of estrogen and progesterone on circadian rhythms in women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02616 01 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) Clinical Aspects of Winter seasonal affective disorder (SAD)		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: N.E. Rosenthal	Environmental Psychiatry Section	CPB/NIMH
Others: C. Brown	Psychologist	CPB/NIMH
D.A. Oren	Senior Clinical Investigator	CPB/NIMH
P. Schwartz	Clinical Associate	CPB/NIMH
J. Giedd	Clinical Associate	CHP/NIMH
S. Swedo	Senior Clinical Investigator	CHP/NIMH
H. Clark	Clinical Social Worker	CPB/NIMH
F. Meyers	Registered Nurse	CPB/NIMH
COOPERATING UNITS (If any) Child Psychiatry Branch, NIMH		
LAB/BRANCH Clinical Psychology Branch		
SECTION Section on Environmental Psychiatry		
INSTITUTE AND LOCATION NIMH Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have previously described the syndrome of winter <u>seasonal affective disorder</u> (SAD), which is characterized by symptoms of <u>depression</u> that recur regularly during the fall and winter months and remit in spring and summer. Although the clinical picture of SAD has been well delineated, little research has been done on its <u>longitudinal course</u>. The condition has been described predominantly in adults, though we and others have reported on a relatively small number of children and adolescents with SAD. While SAD patients are generally considered to be highly sensitive to changes in their physical environment, most notably decreased environmental light, the possible pathogenic role of psychological stressors has been less well explored. </p> <p> A follow-up study of 23 adult patients, discharged from our clinic on average 6 years ago revealed that 16 (70%) patients remained "exclusively seasonal.". Thirteen (57%) continued to use <u>light therapy</u> regularly and successfully each winter after an average of eight years since the diagnosis of SAD had been made. All patients continued to experience worsening of their moods during the winter months, most often in February, and all continued to endorse, and remain invested in, the construct of SAD. </p> <p> Phone interviews with six of the seven <u>children</u> and <u>adolescents</u> with SAD previously studied indicated that all continued to experience some form of seasonal dysfunction, although knowledge about the condition has enabled them to deal effectively with the winter. Five had light boxes in their homes, which they used in an "as needed" and unstructured manner. All reported making conscientious efforts to spend time outdoors during the winter and developed outdoor hobbies. Two reported great benefit from <u>fluoxetine</u>. Patterns of symptomatology for individual cases remained quite stable over time. </p> <p> Evaluation of <u>stress</u>, <u>cognitive appraisal</u> and <u>coping</u> in 37 SAD patients revealed that depressive episodes in SAD patients were not generally precipitated by major <u>life events</u>. SAD patients reported more frequent and severe minor daily stressors than 29 normal controls during the winter when they were depressed. SAD patients did not differ from controls or across seasons in their evaluation of the significance of stressful events or their perception of available resources and coping options. When they were depressed, however, SAD patients did use coping strategies typical of depressives in general, namely those involving more avoidance, regulation or discharge of affect and less problem solving. </p>		

Clinical Aspects of Seasonal Affective Disorder (SAD)

Project Description and Methods:

We have previously described the syndrome of winter seasonal affective disorder (SAD), which is characterized by symptoms of depression that recur regularly during the fall and winter months and remit in spring and summer. SAD can affect adults, children and adolescents. Although there are extensive clinical descriptions of this condition, very little work has been done on its long-term course: whether seasonal mood problems stay seasonal or get better or worse over time. While it is generally accepted that seasonal changes in mood and behavior are influenced by meteorological variables, particularly environmental light, the possible role of psychosocial stressors in the development of symptoms has not previously been explored. Exposing patients with SAD to bright environmental light can reverse their winter symptoms (see Report ZO1 MH 02402 - 01 - CPB). Although these antidepressant effects have been convincingly demonstrated in controlled studies by both ourselves and others, there have been no systematic studies to date of whether the efficacy is retained over time and whether such treatment influences the course of the condition. In this report, we discuss our attempts to address some of these unresolved questions in three studies: (1) follow-up study of adult patients with SAD; (2) follow-up study of child and adolescent patients with SAD; and 3) study of changes in psychosocial stressors, cognitive appraisal and coping in winter and summer in adult patients with SAD.

1. The adult follow-up study: 68 patients with SAD, who had been studied in the NIMH Seasonality Studies Program before April, 1985, were selected for follow-up. Information about mood cycles, life events, treatment and outcome was obtained by means of a questionnaire and several semi-structured interviews. Collateral information was obtained from other therapists. Patients were categorized into seasonal, complicated seasonal and non-seasonal types, based on their overall clinical course and presentation at the conclusion of the study.

2. Child and adolescent follow-up study: About seven years ago we described seven children and adolescents with SAD. For the present study, we have contacted and obtained informed consent from all seven patients. We are planning to evaluate patients by means of structured and semi-structured interviews and to interview parents by phone.

3. Stress, cognitive appraisal and coping in SAD: 37 SAD patients and 29 normal controls were evaluated on indices assessing life stressors, cognitive appraisal and coping during both winter and summer to determine whether or not psychosocial factors such as major life events and minor stressors had an interactive effect on the onset and remission of depressive symptoms. Additionally, two processes - cognitive appraisal and coping - which have been identified as mediators of stressful events and have been found to differ between depressed and non-depressed populations were examined to determine whether SAD patients retained distorted cognitions and dysfunctional coping styles even when depressive symptoms had remitted.

Findings to Date:

1. The adult follow-up study: To date, data are available on only 23 of the 68 selected patients. Patients had been followed in our clinic for two years, on average, and had been discharged from the clinic on average six years before the study was conducted. Sixteen out of 23 (70%) had remained "exclusively seasonal," in that their depressive episodes had continued to be confined to the winter months or they had only used treatment at that time since being discharged from the program. February was the peak month for major depressive episodes. Thirteen out of 23 (57%) continued to use light treatment regularly and successfully each winter after an average of eight years since the diagnosis of

SAD had been made. Bipolar II patients tended to require antidepressant medications in addition to lights. All patients continued to experience worsening of their moods during the winter months, and all continued to endorse, and remain invested in, the construct of SAD.

2. Child and adolescent follow-up study: Results of phone interviews with six of the seven patients indicated that all continued to experience some form of seasonal dysfunction, although knowledge about the condition enabled them to deal effectively with the winter. Five of the subjects had light boxes in their homes and used them in an "as needed" and unstructured manner. All reported making conscientious efforts to spend time outdoors during the winter and took up outdoor hobbies, such as gardening, hiking and water skiing. Two patients reported great benefit from fluoxetine, 20-40 mg per day. Patterns of symptomatology for individual cases remained quite stable over time.

3. Stress, cognitive appraisal and coping in SAD. Results of this study indicate that depressive episodes in SAD patients are not generally precipitated by major life stressors. SAD patients showed no seasonal variation in the number of major life stressors reported and did not differ significantly from normal controls in the number of major life events reported on initial evaluation during the winter. SAD patients did, however, report greater frequency and severity of minor daily stressors than controls during the winter when they were depressed, but not during the summer after they had remitted, though they reported these stressors as more severe than controls even in summer. Examination of cognitive processes involved in coping with stressful events showed no seasonal differences in SAD patients or differences between SAD patients and controls in how they evaluated the significance of stressful events or their perception of available resources and coping options. Finally, like other depressed populations, SAD patients tended to use coping strategies which involved more avoidance, regulation or discharge of affect and less problem solving when depressed. They did not differ from controls, however, when symptoms had remitted.

Significance to Biomedical Research and to the Program of the Institute:

1. The adult follow-up study: The study thus far shows strong support for the stability of the diagnosis of SAD, and for the continued efficacy of light treatment for most patients with SAD. These observations have implications for the validity of the syndrome, which has been incorporated into DSM-III-R as "seasonal pattern", a term that might be used to modify all forms of recurrent mood disorders. They may be value for clinicians and patients interested in learning the degree to which light therapy can be relied upon and clinically effective over time. The fact that a large minority of subjects stopped using light therapy on an ongoing basis may imply that it is not universally helpful in the long run or may reflect the inconvenience of ongoing treatment, as it is currently administered (See report # ZO1 MH 02402 01 CP).

2. Child and adolescent follow-up study: Results obtained so far suggest that SAD as it appears in childhood and adolescence, is a stable condition that manifests itself symptomatically year after year. This underscores the importance of early and accurate detection in order to prevent seasonal depressions, which are impair the child's quality of life and can impede emotional and intellectual development. The finding that most of the children continued to use light therapy in some form and to modify their exposure to natural sunlight by undertaking outdoor activities, suggests the ongoing benefit of these interventions. On the other hand, in at least two children, these measures were not sufficient and they obtained considerable symptomatic relief from the antidepressant fluoxetine.

3. Stress, cognitive appraisal and coping in SAD. Results of this study confirm our earlier observations that depressive symptoms in SAD patients are not triggered primarily by psychological stressors or precipitants. Insofar as SAD patients present with

so-called atypical vegetative symptoms, such as overeating, oversleeping and weight gain, they have been regarded as suffering from "atypical" depressions. Their normal levels of reactivity to psychological stressors distinguishes them from the "atypical" depressives described by Liebowitz and Klein, who have been reported to be exquisitely sensitive to psychosocial stressors such as rejection. The absence of such hypersensitivity in SAD patients reinforces earlier ideas that their depressions are precipitated primarily by the effects of the physical rather than the psychological environment.

Proposed Course:

1. The adult follow-up study: The study is ongoing and should be completed in the forthcoming year.

2. Child and adolescent follow-up study: This particular follow-up study should be completed in the forthcoming year. Our findings thus far have indicated the importance of learning more about SAD in children and adolescents. There is very little in the literature on this topic and it would be important to document the characteristics and prevalence of seasonal changes in mood and behavior and its response to treatment. We are planning studies to investigate these particular questions in collaboration with researchers in the Child Psychiatry Branch, IRP, NIMH.

3. Stress, cognitive appraisal and coping in SAD: This study is regarded as completed and no further data acquisition is planned at this time.

Publications:

Yoney TH, Pigott TA, L'Heureux F, Rosenthal NE. Seasonal variation in obsessive compulsive disorder: Preliminary experience with light treatment. *American Journal of Psychiatry*, 148:12, 1727-1729, 1991.

Rosen LN, Rosenthal NE. Seasonal variations in mood and behavior in the general population: A factor-analytic approach. *Psychiatry Research*, 38: 271-283, 1991.

Wehr TA, Giesen HA, Schulz PM, Anderson JL, Joseph-Vanderpool JR, Kelly KA, Kasper S, Rosenthal NE. Contrasts between symptoms of summer depression and winter depression. *Journal of Affective Disorders*. 23: 173-183, 1991

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02617-01 CP
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 Characters or less. Title must fit on line between the borders.) Restoration of circadian rhythmicity by suprachiasmatic tissue grafts		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Wallace C. Duncan Research Psychologist CPB/NIMH Others: David C. Klein Chief, Section on Neuroendocrinology, LDN, NICHD Norio Ozaki Fogarty Fellow, CPB, NIMH Kathy Michels Research Biologist, LCS, NIMH Thomas A. Wehr Chief, Clinical Psychobiology Branch, NIMH		
COOPERATING UNITS (If any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH Bethesda, Maryland 20892		
TOTAL MAN-YEARS: .50	PROFESSIONAL: .25	OTHER: .25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Daily biological rhythms are controlled by a biological clock located in the <u>suprachiasmatic nucleus</u> (SCN) of the hypothalamus. Thus, lesions of the SCN abolish circadian rhythms in motor activity, wheel-running, cortisol, melatonin, body temperature, etc. Further strong evidence that the SCN contains the daily biological clock is provided by the observation that fetal SCN <u>tissue grafts</u> restore circadian rhythms of wheel-running in SCN-lesioned host animals. Since the fetal SCN graft restores both the circadian oscillation and the behavior driven by the oscillation, not only the clock but also new connections between the clock and sites directly controlling the measured behavior, have been established. The purpose of this project is to investigate a) which rhythms (e.g. body temperature, hormonal, behavioral) are restored by SCN grafts and b) the time course of the restoration.</p> <p>Previous experiments focused on a single clock driven process (e.g. wheel-running or sleep-wake) to assess the success of the SCN graft. In our experiments we will simultaneously measure multiple (behavioral, physiological, neuroendocrine) rhythms of distinct origin in order to determine the extent to which SCN grafts restore outputs of the circadian system (e.g. wheel-running, brain temperature, and <u>melatonin</u>). In early SCN graft experiments, there was difficulty distinguishing between the restoration of a circadian oscillation by donor tissue, and re-expression of the host circadian oscillation as a result of an incomplete SCN lesion. Since the circadian period of the heterozygous "tau mutant hamster" has an abnormally short period of twenty-two hours, we will transplant SCN tissue from the tau mutant hamster into SCN-lesioned wild-type hamsters. The expression of a twenty-two hour rhythm will be used as a marker of a successful SCN graft. Knowledge gained from this experiment will be valuable in examining the role of SCN efferents in the restoration of circadian rhythmicity by SCN grafts, as well as their role in the control of circadian rhythmicity by intact SCN.</p> <p>In the past year we have developed the methodology necessary to continuously and simultaneously monitor <u>circadian</u> rhythms in <u>body temperature</u>, motor activity, urinary <u>corticosteroids</u>, and urinary melatonin in an individual hamster. This methodology will be applied in the next year to investigate the functional restoration of circadian rhythms in SCN-lesioned hamsters that have received SCN grafts.</p>		

Project Description:

The central circadian pacemaker in mammals is located in the suprachiasmatic nucleus of the hypothalamus. Lesions of this nucleus abolish circadian rhythms in motor activity, wheel-running and hormones. In SCN-lesioned animals, SCN grafts restore some rhythms (i.e. wheel-running, sleep-wake), but the diversity of transplanted rhythms has not been examined. The purpose of this project is to investigate a) which rhythms are restored by SCN grafts, and b) the time-course required for this restoration.

Methods:

1) Experimental equipment

Wheel-running in Syrian hamsters is monitored using a laboratory computer as described in project report Z01 MH 02294-01 CP.

A metabolism chamber has been modified in order to a) continuously record brain or peritoneal temperature using radiotelemetry b) continuously monitor motor activity, c) provide Syrian hamsters with liquid diet (calorically similar to the NIH-07 chow formulation) in order to increase urine production, and d) continuously collect urine for subsequent analysis of melatonin and/or corticosteroids.

Findings to Date:

1) Simultaneous Monitoring of Brain Temperature and Motor Activity in a Metabolism Chamber

Preliminary studies indicate that brain temperature and motor activity can be successfully monitored from Syrian hamsters housed in a modified metabolism chamber equipped with a customized receiving antenna. This equipment will be utilized in further studies to examine the restoration of these rhythms in SCN lesioned hamsters.

2) The Effects of Liquid Diet on Urine Production of Syrian Hamsters

The normal daily volume of urine production in Syrian hamsters is between 6-10 ml, a volume which is insufficient to conduct multiple urinary hormone assays over the course of twenty-four hours. Preliminary experiments indicate that liquid diet boosts daily urine production to 60-90 ml. Liquid diet access is a practical method for increasing urine production and collecting multiple endocrine samples during the course of twenty-four hours.

Significance to Biomedical Research:

Restoration of circadian rhythmicity by SCN-grafts in SCN-lesioned hamsters has established the central importance of the suprachiasmatic nucleus in generating a primary circadian oscillation that controls behavioral rhythms, such as wheel-running or sleep-wake behavior. Although it is generally accepted that SCN lesions abolish rhythms of diverse neural and neuroendocrine origins (wheel-running, body temperature, cortisol, melatonin), the diversity of the rhythms re-established by an SCN graft following a complete SCN lesion has not been examined. Knowledge gained from this experiment will be valuable in examining the role of SCN efferents in the restoration of circadian rhythmicity by SCN grafts, as well as their role in the control of circadian rhythmicity by the intact SCN.

Proposed Course:

During the next year the equipment developed thus far will be used to monitor circadian rhythms in motor activity, body temperature, urinary melatonin and urinary cortisol in SCN-lesioned and SCN transplanted, host animals.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00274-18 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Methods of Ionization in Mass Spectrometry		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: S.P. Markey C.F. Ijames J.T. Simpson T.-c. L Wang R.L. Boni	Chief NRC Research Associate Chemist Visiting Fellow Visiting Associate	SAB, LCS, NIMH SAB, LCS, NIMH SAB, LCS, NIMH SAB, LCS, NIMH SAB, LCS, NIMH
COOPERATING UNITS (if any) Laboratory of Chemical Physics, NIDDK, NIH; Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, TN		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Analytical Biochemistry Section		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 3.3	PROFESSIONAL: 2.3	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided)		
<p> An external atmospheric pressure <u>electrospray</u> ionization source and electrostatic lenses for ion transport have been designed, constructed and tested for an <u>ion cyclotron resonance spectrometer</u>. Ions formed at atmospheric pressure have been transmitted into an analyzer cell region, with the resulting demonstration of high mass resolution for both thermally ionized and electrosprayed ions. Improvements in the electrospray source as well as the transport ion optics are in progress, so that the resulting system can be used to measure the molecular weights of proteins and DNA fragments. Derivatization chemistry and separation conditions suitable for the detection of acidic <u>tryptophan</u> metabolites generated in cultured cell or tissue incubation experiments have been demonstrated using liquid chromatography-particle beam-negative chemical ionization mass spectrometry. A new derivatization reagent (pentafluorophenyl boronic acid) has been tested for use in forming cyclic boronates of glycols for improved detection in gas chromatography-mass spectrometry. Organic ion imaging using tandem mass spectrometry has been demonstrated to produce spatially resolved mass selective images, representing a new type of molecular or <u>chemical microscopy</u>. </p>		

Other Professional Personnel Engaged on Project:

Peter J. Todd	Collaborator	Research Scientist, Oak Ridge Natl. Lab., Oak, Ridge, TN
Herman Ziffer	Collaborator	Laboratory of Chemical Physics NIDDK, NIH

Project DescriptionObjective:

Improvement in the specificity and detectability of organic compounds in complex biological matrices requires new developments in mass spectrometric instrumentation and derivatization chemistry. Electrospray ionization and particle beam transport are two means of introducing organic compounds for mass analysis compatible with liquid chromatographic separation techniques, and the requirement for analyzing high molecular weight and polar molecules. Fourier transform ion cyclotron resonance spectroscopy (FT-ICR) is ideal for high sensitivity and high mass analyses, but requires coupling with an external ionization source for efficient sample introduction. Sample introduction into a quadrupole mass analyzer via particle-beam interface coupled to a liquid chromatograph offers many of the advantages of gas chromatography-mass spectrometry transferred to the liquid phase. However, in order to realize these advantages, derivatization methods and separations of derivatized compounds are required for each class of compounds. Even for gas phase analyses, there is a need for new derivatives which are specific for certain function groups, and confer properties which enhance thermal stability, chromatographic properties, ionization and detection. A long-term collaborative project (Oak Ridge National Laboratory) to develop an organic ion imaging spectrometer has reached the stage where biological tissues are being examined, and efforts will be continued to relate these images to known neurochemistry in brain, cultured cells, and other selected tissues. Questions such as the distribution and localization of various kynurenine pathway metabolites can be addressed directly because the use of stable isotope labeled precursors combined with specific mass detection permits the mapping of one metabolite in the presence of the precursor and other metabolites. The common objective of these studies is to improve the mass measurement tools available to the neuroscientist.

Methods Employed:

Mass spectrometric instrumentation or components are designed, built, modified or purchased as required to meet the above objectives. Chemicals are purchased or synthesized as required.

Major Findings:

Electrospray - FT/ICR: The transfer of ions created at atmospheric pressure into an ICR cell operating at 10^{-8} torr in a 3 tesla magnetic field places significant constraints upon experimental apparatus. Previously, we reported the design and construction of an electrospray ion source and transport optics. The entire assembly has been tested with both thermally generated potassium ions and electrosprayed organic ions in order to determine conditions for efficient ion transfer and trapping for mass analysis. The present unit transports approximately 10 per cent of the ions produced in the external source through the cells, and provides a pressure ratio of ~ 1400 and ~ 500 between the external source chamber and the first cell region, and between the first and second cell regions, respectively. Electrosprayed ions were trapped and mass analyzed in the first cell using accumulated trapping. By manually closing the gate valve after 30 seconds of ion trapping, the lower pressure in the ICR cell permitted a resolution of 14,000 FWHH to be measured at m/z 516. Thus, ^{13}C isotope peaks could be readily separated and the charge state directly determined for ions in the spectrum of PEG 1000. These promising results confirm that FT/ICR can be successfully employed to provide

high mass resolution for analysis of electrosprayed ions, and suggest that improvements such as an electronically controlled gate valve, lenses for an off-axis electrospray, or trapping techniques which permit use of the dual cell ICR can be profitably employed and should result in performance benefits which exceed alternative instrumentation.

Liquid chromatography-particle beam-negative chemical ionization mass spectrometry: Conditions for the successful phase transfer catalyzed derivatization of tryptophan and kynurenine pathway metabolites were determined, and pentafluorobenzyl ester formation was found to yield derivatives with excellent properties for negative chemical ionization mass spectrometry. Normal phase liquid chromatographic separation conditions using organic solvents were found, because reverse phase separations were slow and incomplete. While the fact that ^{18}O labeled isotopomers could be formed of each of the carboxylic acid kynurenine metabolites by acid catalyzed exchange has been convenient for quantification, it was necessary to separate $^2\text{H}_4$ -kynurenine from cultured cells exposed to deuterium labeled tryptophan in order to have a unique mass standard. Standard curves for tryptophan and kynurenine were linear over the range 1 to 100 ng/sample. The developed method was used to quantify the production of $^{13}\text{C}_6$ -kynurenine from $^{13}\text{C}_6$ -tryptophan by cultured cells and tissue slices. The 48 hour conversion of $^{13}\text{C}_6$ -tryptophan was measured and determined to increase from 0.8% to 55% of the substrate when challenged with gamma-interferon.

Derivatization Chemistry: Previously, an alternative derivatization reagent (1,1,1,7,7,7-heptafluoroheptan-4-one, HFH) was synthesized in order to have a symmetrical aliphatic ketone which exhibited the reactivity of acetone, but which offered the electron capturing properties of hexafluoroacetone. HFH reacted with 1,2-diols or 1,2-amino alcohols in the presence of acid catalysts to form cyclic derivatives, but the relatively harsh conditions required for cyclization, and the lack of sensitivity enhancement for negative chemical ionization mass spectrometry precluded the routine applicability of this reagent to biological problems. A new reagent, pentafluorophenylboronic acid (FPBA), was synthesized and tested with regard to its reactivity and the properties of the resulting derivatives. FPBA reacted at room temperature in dichloromethane with simple diols as well as methyl 2-methylglycerate, and 3,4-dihydroxyphenylethylene glycol. The resulting derivatives were stable when kept anhydrous, had excellent gas chromatographic properties, and good electron ionization properties. The negative chemical ionization spectra of simple diols-FPBAs did not exhibit greater sensitivity than electron ionization. However, in the case of the di-FPBA derivative of 3,4-dihydroxyphenylethylene glycol, the signal enhancement with negative chemical ionization was significant (30-to-50 fold). These derivatives will be further characterized with other polyfunctional substrates of biological importance.

Organic ion imaging: A triple quadrupole mass spectrometer has been interfaced successfully with a wide-angle secondary ion microprobe by a group of collaborating scientists at Oak Ridge National Laboratory using components initially assembled at NIH. The combination permits acquisition of data necessary to determine the distribution of targeted organic analytes even in the presence of overwhelming isobaric interference. Micrographs generated from secondary ion intensity alone were compared to those generated using secondary ionization with tandem mass analysis, both for image reference and to show improvement in image quality that can be attained by this instrumentation.

Significance to Biomedical Research:

Structure elucidation of unknown compounds in complex mixtures, or the specific detection and quantification of known compounds and their isotopic variants remain important areas of biomedical research. Polar, non-volatile compounds are frequently encountered in neurochemistry, and the ionization methods and instrumentation and derivatization chemistry being developed are particularly relevant to the analyses of these materials.

Proposed Course:

The electrospray ion source on the FT/ICR spectrometer will be further improved in order to permit the efficient trapping of electrosprayed ions from polar organics with the high sensitivity and high resolution inherent to this instrument. Having proven that electrostatic lenses can be used to guide externally generated ions into the FT/ICR cell, the next task will be to utilize electrospray techniques which generate more intense ion currents, and modify the transmission optics to reduce the flux of neutrals into the high vacuum system. The testing of pentafluorophenylboronates of diols will be continued, using comparison with known cyclization derivatives of compounds of importance to related projects in the laboratory. The liquid chromatographic-particle beam-negative chemical ionization mass spectrometric analysis of biological extracts containing kynurenines (Z01 MH02384-05) will continue to be applied to understanding the kinetics of metabolism of tryptophan in cell culture media and brain tissues. Organic ion imaging of cultured cells and tissues will be explored. Because this is essentially a new form of molecular histology, efforts will be directed toward sample preparation, with emphasis on comparing frozen and dried samples with those stained with specific reagents.

Publications:

Ijames CF, Markey SP. Design of an electrospray ionization source for Fourier transform mass spectrometry. In: Proceedings of the 39th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, Nashville, 1991, 1548-9.

Markey SP, Simpson JT, Sherman R, Ziffer H. 1,1,1,7,7,7-hexafluoroheptan-4-one: a new derivatization reagent for GC/MS of diols. In: Proceedings of the 39th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, Nashville, 1991, 1045-6.

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Ijames CF, Markey SP. An external source for Fourier transform mass spectrometry. In: Proceedings of the 40th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, Washington, 1992, in press.

Boni RL, Simpson JT, Saito K, Markey SP. Phase transfer catalyzed derivatization of L-tryptophan and L-kynurenine and their quantification by particle beam LC-NCI MS. In: Proceedings of the 40th American Society for Mass Spectrometry Conference on Mass Spectrometry and Allied Topics, Washington, 1992, in press.

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Pike SE, Markey SP, Ijames C, Jones KD, Tosato G. A new role for lactic acid: autocrine B cell growth stimulation. Proc Natl Acad Sci 1991; 88:11081-5.

Todd PJ, Short RT, Grimm CC, Holland WM, Markey SP. Organic ion imaging using tandem mass spectrometry. Anal Chem 1992, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00279-10 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Pharmacology of Neurotoxins		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.: S.P. Markey C.J. Markey J.T. Simpson T.-c. L Wang J. Rodriguez	Chief Guest Worker Chemist Visiting Fellow Visiting Fellow	SAB, LCS, NIMH SAB, LCS, NIMH SAB, LCS, NIMH SAB, LCS, NIMH SAB, LCS, NIMH
COOPERATING UNITS (if any) Lab Experimental Pathology, NCI, NIH Biomedical Mass Spectrometry Unit, University of New South Wales, Sydney, Australia		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Analytical Biochemistry Section		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 2.4	PROFESSIONAL: .8	OTHER: 1.6
CHECK APPROPRIATE BOX(ES) _ (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues _ (c) Neither _ (a1) Minors _ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)		
<p>General mechanisms of neurotoxicity have been investigated over the past several years, principally using the parkinsonian syndrome producing toxin <u>1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)</u> and its analogues. Studies have been completed assessing the occurrence of compounds structurally related to MPTP or its major metabolite 1-methyl-4-phenylpyridinium (MPP+) in post-mortem brain tissue from patients with idiopathic <u>Parkinson's</u> disease. An immunoassay procedure was developed for the detection of MPP+, and shown to be very sensitive in tissues from animals that had been exposed to MPTP or one of its analogs. Based upon a survey of immunoactivity in extracts of several regions of normal human control brain tissue, there was no evidence for increased immunoactivity in Parkinson's disease patients. Thus, there is no evidence for an environmental neurotoxin chemically related to MPTP in the pathogenesis of idiopathic Parkinson's disease.</p> <p>Several lines of evidence have suggested that <u>hydroxyl radicals</u> generated either by redox cycling a toxin utilizing endogenous enzymes, or as a result of metabolism which has been activated by a toxin, may be involved in the pathogenesis of neurodegenerative disorders. Hydroxyl radical damage may effect many different cell constituents. However, oxidative damage to neuronal DNA could result in impaired neuronal function if the damage remained unrepaired and accumulated. In order to measure oxidative damage to neuronal or mitochondrial DNA, gas chromatographic-mass spectrometric methods are being developed for the detection of <u>thymine glycol</u>, one of the oxidation products of thymine. The method releases methyl 2-methylglycerate from double stranded DNA by several chemical steps, prior to derivatization for mass spectrometric detection.</p>		

Other Professional Personnel Engaged on this Project:

Thomas J. Sobotka, Senior Investigator, Division of Toxicology, CFSAN, FDA
Jan Johannessen, Senior Investigator, Division of Toxicology, CFSAN, FDA

Objectives:

Two projects have been directed toward elucidating the modes of action and understanding the consequences of neurotoxins, especially as they relate to disease states in man. First, the well-documented neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in man and in non-human primate and resultant severe parkinsonism raises the questions of how this neurotoxin works, and of whether similar environmental or endogenously generated neurotoxins may contribute to this progressive neurodegenerative disease. We have sought to evaluate whether MPTP or MPTP-like compounds possess properties one would predict for such toxins, and whether there is any evidence for their presence in the environment. Secondly, studies of the effect of neurotoxins upon DNA repair processes have been initiated due to the apparent common molecular mechanisms of several progressive neurodegenerative disorders associated with aging.

Methods employed:

MPTP toxicity is being studied by: qualitative and quantitative observations of animal behavior and locomotion; neurochemical determination of catecholamines and their metabolites in specific brain regions by high performance liquid chromatography with electrochemical detection (HPLC-EC); determination of the pattern of MPTP distribution, metabolism, and excretion using radio and stable isotope labeled MPTP; autoradiography and organic ion imaging of tissue exposed to labeled MPTP; and mass spectrometry of isolated products. MPTP and MPP⁺ analogues have been quantified by immunochemical methods using antibodies raised to amino-analogues prepared earlier in these studies.

Gas chromatographic and mass spectrometric methods are being developed and tested for the quantification of thymine glycol, as a product of DNA oxidation.

Major findings:

MPTP is a systemically active neurotoxin which kills catecholamine containing cells, primarily the dopaminergic neurons comprising the nigrostriatal system. One goal of this program has been to search for neurotoxins structurally related to MPTP or its toxic metabolite in the pathogenesis of Parkinson's disease. The toxic actions of MPTP are dependent on two steps, monoamine oxidase (MAO) mediated oxidation to the pyridinium form, 1-methyl-4-phenylpyridine (MPP⁺), and active sequestration of this metabolite within catecholaminergic terminals via the catecholamine uptake system. Blocking either one of these steps prevents the toxic effects of MPTP. A number of MPTP analogs have been synthesized and tested in order to define the structural requirements for toxicity. Several of these analogs were useful as antigens when diazotized and cross-linked to bovine serum albumin. The resulting rabbit antibodies have been used to develop an immunoassay, and then to determine cross-reactivities of thirty-three structurally related compounds, and to define the sensitivity and chemical reactivity of the polyclonal antibodies. Two extraction procedures were then developed which would be sufficiently general as to concentrate compounds structurally related to either MPTP or its pyridinium metabolite MPP⁺. The extraction procedures and the immunoassay were combined and applied to tissues from animals exposed to MPTP, and indicated detectability of MPP⁺-immunoreactivity in monkey brain greater than eight weeks after exposure. No difference in

immunoreactivity was measureable in extracts from human brains of Parkinson's disease patients or controls, and particularly low levels of immunoactivity were found in the striatum relative to the levels measured in several cortical regions. From these studies, there is no evidence for the role of an environmental neurotoxin chemically related to MPTP in the pathogenesis of Parkinson's disease.

Research on analytical methods suitable for the sensitive detection of oxidized thymine (thymine glycol) in intact double stranded DNA have been continued. The present scheme uses hydrolysis, borohydride reduction, and acidic methanolysis to release 2-methylglyceric acid methyl ester specifically and quantitatively from thymine glycol. The chemistry and detection of this product has been improved such that picogram amounts of thymine glycol can be measured, but this requires hundreds of micrograms of DNA. Considerable attention to avoiding artifactual formation of thymine glycol during sample analysis is required, and methods continue to be refined with regard to derivatization chemistry and mass spectrometric analysis.

Significance to Biomedical Research:

The MPTP-lesioned primate has been proven to be a most useful animal model of idiopathic Parkinson's disease in man. The mechanism of action of this and related neurotoxins may be relevant to the human disease process, and attempts to identify neurotoxic environmental agents may lead to effective preventative measures for a common disease of aging. Methods of measuring oxidized DNA bases may be particularly useful chemical monitors for human exposure to neurotoxins which cause oxidative damage, particularly to DNA repair enzymes.

Proposed Course:

Most of the studies with MPTP have now been completed in this laboratory, although some collaborative work with regard to long term exposure studies is being continued. The analysis of thymine glycol, one product of DNA oxidation, is being investigated with respect to neurodegenerative disorders. Future efforts will concentrate on improving and applying the present methods to DNA isolates from human and animal tissues, and to the development of more sensitive negative chemical ionization methods for intact (rather than chemically degraded) thymine glycol.

Publications:

Ikeda H, Markey CJ, Markey SP. Search for neurotoxins structurally related to 1-methyl-4-phenylpyridine (MPP+) in the pathogenesis of Parkinson's disease. Brain Research 1992;575:285-298.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02384-06LCS
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT <small>(88 characters or less. Title must fill an entire line between the borders)</small> Brain Quinolinic Acid Metabolism: Role in Neuropathology		
PRINCIPAL INVESTIGATOR <small>(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)</small> P.I.: Melvyn P. Heyes, Visiting Scientist, SAB, LCS, NIMH Others: Sanford P. Markey, Chief, SAB, LCS, NIMH Riccardo L. Boni, Visiting Associate, SAB, LCS, NIMH Kuniaki Saito, Visiting Fellow, SAB, LCS, NIMH Dmitry Naritsin, Visiting Fellow, SAB, LCS, NIMH Juan Rodriguez, Visiting Fellow, SAB, LCS, NIMH		
COOPERATING UNITS <small>(if any)</small> Sect. Clin. Pharm. (I.N. Mefford), ETB, NIMH; Sect. Histopharm. (D.M. Jacobowitz), LCS, NIMH; Lab Neuropath. Neuroanat. (S. Nadi, T. Nowak); Lab Chem. Phys. (H. Ziffer), NIDDK; Pediatric Branch, NCI (P. Brouwers, P. Pizzo); Veterinary Res. Branch, NINDS (E.K. Jordon).		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Analytical Biochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, MD		
TOTAL STAFF YEARS: 6.3	PROFESSIONAL: 3.6	OTHER: 2.7
CHECK APPROPRIATE BOX(ES) _ <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither _ <input type="checkbox"/> (a1) Minors _ <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <small>(Use standard unreduced type. Do not exceed the space provided)</small> <p> <u>Quinolinic acid</u> (QUIN) is an excitotoxic tryptophan and kynurenine pathway metabolite that has been implicated in the etiology of many neurologic disease. We have discovered that the most pronounced increases in brain and cerebrospinal fluid QUIN levels occur in patients with <u>inflammatory neurologic diseases</u>, including patients infected with the <u>human immunodeficiency syndrome</u>. The mechanisms involved in increasing QUIN synthesis from L-tryptophan include induction of <u>indoleamine-2,3-dioxygenase</u>, <u>kynurenine-3-hydroxylase</u>, <u>kynureninase</u> and <u>3-hydroxyanthranilate-3,4-dioxygenase</u>. <u>Macrophages</u> have a high capacity to convert L-tryptophan to QUIN and such cells may be a predominant source of QUIN in the central nervous system of patients with inflammatory neurologic diseases. Strategies to attenuate the synthesis of QUIN or attenuate its stimulatory effects on the <u>N-methyl-D-aspartate receptors</u> which mediate its neurologic effects are potentially new approaches to the <u>therapy</u> of inflammatory neurologic diseases. </p>		

Objectives

Key metabolites of the kynurenine pathway are neuroactive within the central nervous system and have been proposed as mediators of neuronal dysfunction and neurodegeneration in a broad spectrum of human neurologic diseases. In particular, we have established that quinolinic acid (QUIN), an excitotoxic N-methyl-D-aspartate receptor agonist, is elevated in patients infected with the human immunodeficiency virus (HIV), the cause of AIDS. The clinical significance of this finding is emphasized by the significant correlations between cerebrospinal fluid QUIN levels and quantitative measures of neuropsychologic impairments. The objectives of our studies have been: 1) To determine whether increases in QUIN occur in brain in other human neurologic diseases in addition to HIV; 2) To identify the immunologic and biochemical mechanisms responsible for increasing QUIN levels within the blood and brain following immune activation; 3) Identify appropriate animal models of the disturbances in kynurenine pathway metabolism following immune activation; 4) Develop strategies to attenuate the formation of QUIN following immune stimulation. 5) Determine whether such strategies attenuate neurologic dysfunction and brain atrophy in inflammatory neurologic diseases.

Methods employed

Highly sensitive, specific and accurate assays based on gas chromatography/mass spectrometry or high performance liquid chromatography have been developed to quantify the concentrations of L-tryptophan, L-kynurenine, kynurenic acid, 3-hydroxykynurenine, anthranilic acid and QUIN in cerebrospinal fluid, brain tissue, various systemic organs and blood. The assays have been adapted to measure the activities of the kynurenine pathway enzymes, indoleamine-2,3-dioxygenase, kynureninase, kynurenine aminotransaminase, kynurenine-3-hydroxylase and 3-hydroxyanthranilate-3,4-dioxygenase in brain and systemic tissues. A monoclonal antibody to human indoleamine-2,3-dioxygenase has also been obtained to determine the localization of indoleamine-2,3-dioxygenase in brain and systemic tissues. Several animal models of brain and/or systemic immune stimulation have been established, including ischemic neuronal injury in gerbils and systemic administration of immune stimuli and cytokines to small rodents. Kynurenine pathway metabolism is also studied in tissue and cell culture.

Major Findings

It is clear that substantial increases in QUIN and other kynurenine pathway metabolites occur in many inflammatory neurologic diseases as well as HIV infection. Clearly QUIN has the potential of mediating neuronal injury in many neuropathologic diseases. We have established that following intracerebral immune activation, particularly where there is infiltration of the brain by macrophages, that the activities of indoleamine-2,3-dioxygenase, kynureninase, kynurenine-3-hydroxylase and 3-hydroxyanthranilate-3,4-dioxygenase are increased, although there may be selectivity of such induction depending on the species studied as well as the severity and localization of immune stimulation. Mice or gerbils given systemic injections of cytokines are an excellent model of kynurenine pathway responses in man where the immune stimulus is systemic, such as in septicemia. Both ischemic neuronal injury in gerbils and poliovirus infection of the spinal cord in rhesus macaques replicate the increases in kynurenine pathway metabolism where the immune stimulus is restricted to the central nervous system. Evidence was obtained that antibodies to interferon- γ can attenuate QUIN

formation following systemic immune activation. Following intracerebral immune activation, indoleamine-2,3-dioxygenase expression is localized in glial cells and macrophage infiltrates.

Significance to biomedical research

A substantial proportion of neurologic diseases are associated with stimulation of the immune system, including the AIDS dementia complex, Lyme disease, head injury and multiple sclerosis. The incidence of the AIDS dementia complex and Lyme disease are likely to continue to increase. In some diseases, no therapy is available and in others, therapies are marginally effective. QUIN was the first and currently only identified neurotoxin that has been shown to be increased in HIV infected patients and correlate with the severity of neurologic deficits. QUIN has the potential of being an important contributor to neuronal dysfunction and injury in patients with inflammatory diseases. Our understanding of the mechanisms responsible for its formation is incomplete and strategies to attenuate its formation and neuropathologic effects are rudimentary. Therefore, further studies of QUIN metabolism in inflammatory neurologic diseases may contribute to alleviating the severity of inflammatory neurologic diseases.

Proposed course

Studies to identify successful strategies to attenuate QUIN formation following immune stimulation in the central nervous system and systemic tissues in appropriate animals models and *in vitro* systems will continue. Clinical studies will determine whether QUIN measures can be used to predict therapeutic responses to anti-retroviral drugs.

Publications

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Heyes MP, Saito K, Markey SP. Human macrophages convert L-tryptophan to the neurotoxin quinolinic acid. Biochem J 1992; 283:633-5.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 00332-14 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Animal Models for the Study of Neuropharmacologic Effects		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) Charanjit S. Aulakh, Ph.D., Staff Pharmacologist, Section on Clinical Neuropharmacology, LCS, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Clinical Neuropharmacology		
INSTITUTE AND LOCATION National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.6	PROFESSIONAL: 1.3	OTHER: 0.3
CHECK APPROPRIATE BOX(ES) _ (a) Human subjects _ (b) Human tissues X (c) Neither _ (a1) Minors _ (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided) <p>By using various 5-HT receptor subtype selective antagonists, we have demonstrated that <u>m-chlorophenylpiperazine</u> (<u>m-CPP</u>, a 5-HT₁ agonist)-induced prolactin secretion is mediated by stimulation of postsynaptic 5-HT_{1C} receptors while corticosterone secretion may be mediated by an antagonistic effect at 5-HT₃ receptors or by non-serotonergic mechanisms. The food intake suppressant effect of the hallucinogenic agent, <u>1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane</u> (<u>DOI</u>) was demonstrated to be mediated by stimulation of both 5-HT_{1C} and 5-HT₂ receptors. In another study, we have demonstrated that <u>clonidine</u> stimulates growth hormone secretion by activation of α_2-heteroreceptors present on 5-HT nerve terminals which, in turn, enhance 5-HT activity by stimulation of post synaptic 5-HT_{1C} receptors to promote growth hormone releasing factor. Furthermore, either 5-HT_{1C} receptors or α_2-adrenergic heteroreceptors or both are functionally subsensitive in the Fawn-Hooded rat strain relative to the Wistar rat strain.</p> <p>In a separate series of experiments, chronic treatment with the <u>tricyclic antidepressants</u> and <u>clorgyline</u> (MAO type A inhibiting antidepressant) decreased the steady state concentrations of G protein α subunit $G_{s\alpha}$ and; to a lesser extent $G_{i\alpha}$ in several brain regions, while $G_{o\alpha}$ was increased by tricyclics but not clorgyline. Chronic treatment with <u>carbamazepine</u> decreased $G_{s\alpha}$ in several brain areas reaching significance in the neostriatum, while chronic <u>lithium</u> treatment had no unequivocal effect. Lithium treatment significantly increased $G_{i\alpha}$ in the hypothalamus and hippocampus, whereas carbamazepine decreased $G_{i\alpha}$ in the frontal cortex. These findings indicate that long-term treatment with antidepressant and antibipolar drugs exert differential effects on G protein α subunits, and that antidepressant or antibipolar efficacy may potentially be based on functional modifications of signal transduction.</p>		

Other Collaborative Professional Personnel Engaged on the Project

D. L. Murphy, M.D.	Chief	LCS	NIMH
J. L. Hill, Ph.D.	Biostatistician	LCS	NIMH
K. M. Wozniak, Ph.D.	Visiting Associate	LCS	NIAAA
K. P. Lesch, M.D.	Visiting Associate	LCS	NIMH
H. T. Yoney, M.D.	Visiting Fellow	LCS	NIMH

Project Description

We have conducted a series of experiments to investigate the role of various 5-HT receptor subtypes in mediating food intake, temperature and neuroendocrine effects of 5-HT agonists. In a separate series of experiments, we have explored the role of G proteins in the modification of signal transduction by chronic antidepressant and antibipolar treatments which might help us understand the molecular mechanisms responsible for both the therapeutic and side effects of these drugs.

Methods Employed

In the food deprivation paradigm, animals were trained to take their daily food (Purina food pellets) from 10:00 AM to 2:00 PM for 10 days before initiation of drug treatment. At the end of the first hour of food access, the remaining food was weighed; the difference from the original amount constituted one measure of food intake.

In neuroendocrine experiments, the animals were sacrificed by decapitation, and trunk blood was collected in centrifuge tubes containing EDTA. Plasma concentrations of prolactin, corticosterone, and growth hormone were measured by radioimmunoassays.

The G protein subunits, $G_{s\alpha}$, $G_{i\alpha}$ and $G_{o\alpha}$ were quantitated in various rat brain regions using enzyme-linked immunosorbent assay (ELISA) techniques. In the antidepressant studies, Imipramine (5 mg/kg/day), clomipramine (5 mg/kg/day), desipramine (5 mg/kg/day) clorgyline (1 mg/kg/day) or saline was subcutaneously administered by means of osmotic minipumps for 28 days; the pumps were reimplanted after two weeks. For lithium and carbamazepine treatments, animals were given rat chow containing lithium carbonate (1.6 g/kg) and carbamazepine (5.0 g/kg) for 28 days.

Major Findings

Administration of various doses of clonidine increased plasma growth hormone levels. Pretreatment with the α_2 -adrenergic antagonists, yohimbine and 1-PP, completely blocked clonidine's effect on growth hormone levels. Pretreatment with the 5-HT₃ receptor antagonist, MDL-72222, the 5-HT_{1A}/5-HT₂ antagonist, spiperone, and the mixed β -adrenergic/5-HT_{1B} antagonists l-propranolol and CGP361A did not attenuate clonidine-induced increases in growth hormone levels. In contrast, pretreatment with the nonselective 5-HT_{1/2} antagonist metergoline and the 5-HT_{1C}/5-HT₂ selective antagonists mesulergine, mianserin and ritanserin significantly attenuated clonidine-induced increases in growth hormone levels. Clonidine administration failed to increase growth hormone levels in the Fawn-Hooded rat strain.

In another study, chronic treatment with tricyclic antidepressants and clorgyline (MAO type A inhibiting antidepressant) decreased the steady state concentrations of G protein α subunit $G_{s\alpha}$ and, to a lesser extent $G_{i\alpha}$ in several brain regions, while $G_{o\alpha}$ was increased by tricyclics but not clorgyline. Chronic treatment with carbamazepine decreased $G_{s\alpha}$ in several brain regions reaching significance in the neostriatum, while chronic lithium treatment had no

unequivocal effect. Lithium treatment significantly increased $G_{i\alpha}$ in the hypothalamus and hippocampus, whereas carbamazepine decreased $G_{i\alpha}$ in the frontal cortex.

Significance to Biomedical Research and Program of the Institute

The demonstration that 5-HT_{1C} receptor subtype is involved in mediating prolactin and growth hormone secretion is important because hormonal responses to serotonergic drugs are frequently used as a means to evaluate brain serotonergic function in humans. Brain serotonin changes have been implicated in the etiology of affective illness and mode of action of antidepressant and antimanic drugs. The demonstration of functional subsensitivity of 5-HT_{1C} receptors in the Fawn-Hooded rat strain suggests that this rat strain may prove to be a useful genetic model for some neuropsychiatric disorders with possible abnormalities in serotonergic function such as depression, obsessive compulsive disorder and the eating disorders. The demonstration of changes in G protein α subunits following chronic treatment with antidepressants and antibipolar drugs suggests that antidepressant or antibipolar efficacy may potentially be based on functional modifications of signal transduction.

Proposed Course

During the next year, we will explore functional adaptational changes in the serotonergic system using 5-HT subtype agonists as challenge agents. With the FH rat strain, we plan to identify other altered behavioral and neuroendocrine responses to serotonergic agents and clarify the biochemical nature of these defects, and also explore functional adaptational changes in the serotonergic system using 5-HT agonists as challenge agents following long-term antidepressant treatment. In addition, we plan to investigate the effects of chronic treatment with imipramine, clomipramine, fluoxetine, clorgyline, lithium and carbamazepine on G protein α_s , α_q , and α_{12} subunit mRNA expression, as well as 5-HT transporter mRNA expression in rat brain.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00336-13 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) The Phenomenology and Treatment of Obsessive-Compulsive Disorder in Adults		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Dennis L. Murphy, M.D., Chief, Laboratory of Clinical Science, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Clinical Neuropharmacology		
INSTITUTE AND LOCATION National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 5.2	PROFESSIONAL: 3.6	OTHER: 1.6
CHECK APPROPRIATE BOX(ES) X (a) Human subjects (b) Human tissues (c) Neither (a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>In studies of obsessive-compulsive disorder (OCD) <u>co-morbidity</u>, 59 OCD patients were found to have a substantial overlap in <u>eating disorder</u>-related symptoms, with intermediate eating disorder inventory scores between patients with anorexia nervosa or bulimia nervosa and healthy controls. Patients with the focal dystonia, <u>blepharospasm</u>, were found to share significantly more OCD-related symptoms than healthy controls.</p> <p>In psychobiologic studies, OCD patients exhibited no differences in arterial plasma <u>catecholamine</u> concentrations compared to healthy controls when both groups were sampled over time in an <u>anxiety</u>-inducing situation — a finding congruent with other data that continue to reveal more <u>serotonin</u>-related abnormalities than other neurotransmitter system differences in OCD patients. In one of the first studies of neuropeptides in this disorder, patients with OCD were found to have abnormalities in the regulation of <u>vasopressin</u> and <u>corticotrophin releasing factor</u> secretion.</p>		

Other Collaborative Professional Personnel Engaged on the Project

T.A. Pigott, M.D.	Medical Officer	LCS	NIMH
T. Grady, M.D.	Sr. Staff Fellow	LCS,	NIMH
F. L'Heureux, M.D.	Visiting Fellow	LCS	NIMH
K. Bihari, M.D.	Visiting Fellow	LCS	NIMH
C. Benkelfat, M.D.	Research Psychiatrist	McGill University,	Montreal, Canada
J.L. Hill, Ph.D.	Biostatistician	LCS	NIMH
T.R. Insel, M.D.	Medical Officer	LCS	NIMH
M.A. Altemus, M.D.	Medical Officer	LCS	NIMH

Project DescriptionObjectives

1. To evaluate the phenomenological and psychobiological features of adults with obsessive-compulsive disorder to better understand the development, course, and treatment of this neuropsychiatric disorder.
2. In addition, to explore the mechanisms of action, side effects, and other characteristics of drugs such as clomipramine, fluoxetine, and buspirone that may have therapeutic effects in obsessive-compulsive disorder.
3. To compare the psychobiological and clinical features of adults with obsessive-compulsive disorder to other psychiatric disorders with obsessive or compulsive symptomatology including trichotillomania, the focal dystonias and anorexia and bulimia nervosa in order to better understand and characterize the spectrum of potential OCD-related disorders.

Methods Employed

Patients are screened and, if suitable, accepted into our adult obsessive-compulsive disorder (OCD) clinic program. Diagnoses are established using DSM-III-R criteria based on a standardized interview schedule including the SCID. Duration of illness, prior treatment, family history, and other relevant information is obtained. After stopping all psychopharmacological treatment, we measure the patient's baseline symptom severity using a number of interview-based and self-rating scales.

During this baseline, medication-free interval, patients are given a number of psychobiological tests. These tests include procedures to determine patient response to various neurotransmitter-selective pharmacological challenges such as two different serotonin (5-HT) agonists, m-chlorophenylpiperazine (m-CPP) and buspirone as well as amphetamine. An LP may be performed to evaluate cerebrospinal fluid concentrations of neurotransmitters, their metabolites and neuropeptides. A sodium chloride infusion may also be performed in order to evaluate the integrity of the neuroendocrine system with particular emphasis upon the neuropeptides arginine vasopressin and oxytocin which have been shown to have important effects on memory and learning. Positron emission tomography under various conditions may also be performed.

Suitable patients are typically admitted into therapeutic trials comparing new drugs with standard drugs such as clomipramine or placebo. Some of the psychobiological tests and ratings obtained at baseline are repeated at various times during drug treatment. Responses to treatment are compared with baseline measures and are correlated with changes in psychobiological measures and plasma levels of drugs.

Major Findings

OCD symptomatology has occasionally been noted in other disorders, but rarely quantified. Two studies completed this year revealed some interesting areas of overlap between OCD and other neuropsychiatric disorders. Dr. Pigott and coworkers administered a structured, self-rating scale, the Eating Disorder Inventory, to 59 outpatients in our obsessive-compulsive disorder clinic and to 50 sex-matched normal volunteers. The Eating Disorder Inventory has been previously validated as a reliable measure of the specific cognitive and behavioral dimensions of the psychopathology typical of patients with eating disorders. The scores of the patients with obsessive-compulsive disorder and of the healthy comparison subjects were compared with those of 32 female inpatients with anorexia nervosa (N=10) or bulimia nervosa (N=22) who had also been given the inventory. The patients with obsessive-compulsive disorder scored significantly higher than the healthy comparison subjects on all eight subscales of the Eating Disorder Inventory: drive for thinness, bulimia, body dissatisfaction, ineffectiveness, perfectionism, interpersonal distrust, interceptive awareness, and maturity fears. Relative to the healthy subjects, male patients with obsessive-compulsive disorder had more symptoms than female patients with obsessive-compulsive disorder. The scores of the female patients with obsessive-compulsive disorder were midway between those of the 32 female patients with eating disorders and those of the 35 female normal subjects. These results suggest that patients with obsessive-compulsive disorder display significantly more disturbed eating attitudes and behavior than healthy comparison subjects and that they share some of the psychopathological eating attitudes and behavior that are common to patients with eating disorders.

In another study, Dr. Bihari and co-workers investigated patients with essential blepharospasm, which is considered to be a form of focal dystonia. Many patients with blepharospasm have been noted to have concomitant depression, anxiety, phobias, hypochondriasis, and other emotional and behavioral disorders, suggesting a psychiatric component to the disease that, overall, has some phenomenological similarities to obsessive-compulsive disorder (OCD) in terms of the repetitive, perseverative, and persistent nature of the symptoms. The Maudsley OCD questionnaire was administered to 21 patients with blepharospasm and 19 normal controls. The blepharospasm patients scored significantly higher than the controls ($p < .01$). Although preliminary, this study does support at least a phenomenological link between OCD and blepharospasm.

Several new psychobiological investigations in OCD patients were completed in the last year. As part of series of investigations concerning elements of anxiety in OCD patients, Dr. Benkelfat and coworkers sampled plasma catecholamines and their metabolites in 13 medication-free patients with obsessive-compulsive disorder and 29 normal controls. In addition to severe OCD symptoms, the patients had significantly higher anxiety, tension, and resting pulse rates than the controls. Nonetheless, mean plasma concentrations of norepinephrine (NE) and epinephrine (E), the catecholamine metabolites 3-methoxy-4-hydroxyphenylglycol (MHPG) and homovanillic acid (HVA), and the stress-related hormone cortisol did not differ between OCD patients and normal controls. When the patients and control populations were combined and average plasma NE and E levels calculated over 35 min, subjects with a higher mean NE output (>1.1 pm/ml) had higher Profile of Mood States depression scores than subjects with a low NE output (<1.1 pm/ml). Altogether, these results indicate that elevated plasma catecholamine measures are not likely to be associated with the pathophysiology of OCD.

In light of prior data that the central administration of vasopressin in animals is associated with abnormal persistence of behaviors acquired under aversive conditioning, Dr. Altemus and coworkers studied the secretion of arginine vasopressin into the cerebrospinal fluid and plasma in patients with obsessive-compulsive disorder and controls. Patients with

obsessive-compulsive disorder were found to have significantly elevated basal levels of arginine vasopressin in the cerebrospinal fluid and significantly increased secretion of arginine vasopressin into the plasma in response to hypertonic saline administration. Moreover, seven of 12 patients with obsessive-compulsive disorder showed a loss of the normal linear relationship between plasma arginine vasopressin level and osmolality. In addition, cerebrospinal fluid corticotropin releasing hormone, which has synergistic effects with arginine vasopressin centrally and at the pituitary gland, was also significantly elevated in patients with obsessive-compulsive disorder compared with controls.

In a controlled study designed to evaluate whether buspirone might have additive beneficial effects with clomipramine, fourteen OCD patients who had received at least three months of treatment with clomipramine were given buspirone in a ten week, double-blind study. Prior to the addition of buspirone, these patients as a group had shown a partial but incomplete reduction (averaging 28%) in OCD symptoms during clomipramine treatment alone. Although adjuvant buspirone treatment was well tolerated in most subjects, OCD and depressive symptoms, as assessed by standardized rating scales, did not significantly change from baseline scores achieved on clomipramine treatment alone, either after the addition of placebo for two weeks or buspirone (60 mg/d) for an additional ten weeks. However on an individual basis, five (36%) of the fourteen patients did have an additional 25% reduction in OCD symptoms after adjuvant buspirone treatment. While this double-blind study did not indicate that buspirone therapy was associated with further significant clinical improvement in OCD or depressive symptoms for the OCD patients as a group, there may be a subgroup of patients who do benefit from adjuvant buspirone therapy.

Significance to Biomedical Research and the Program of the Institute

We have previously reported an increase in OCD symptoms in response to the 5-HT agonist, m-CPP, in OCD patients compared to controls. Behavioral hyperresponsivity in OCD patients has not previously been seen with single doses of other psychoactive drugs that have activating or anxiogenic properties, such as yohimbine, sodium lactate, or d-amphetamine. The lack of a differential plasma catecholamine change in OCD patients compared to controls in an anxiety-provoking situation is in keeping with the lack of differential responses to non-5-HT challenge agents, including, in particular two catecholamine system stimulating agents, yohimbine and amphetamine.

The abnormalities in vasopressin concentrations in OCD patients are a novel finding, and are of theoretical interest in light of data in experimental animals that the acute peripheral and central administration of arginine vasopressin delays the extinction of behaviors acquired during aversive conditioning. It has been postulated that peripherally administered vasopressin influences memory function through its arousing effects on blood pressure and other physiologic variables, producing visceral afferent signals to cause central release of vasopressin, while central vasopressin administration may act directly via the activation of disparately located central vasopressin receptors in response to osmotic stimulation. Increased activity of both the vasopressin and CRF stress-responsive, arousal-producing systems might conceivably contribute to several clinical features of obsessive-compulsive disorder, including perseverative behaviors, a narrow focus of attention and exaggerated grooming behaviors.

The lack of an additive effect of buspirone combined with clomipramine suggests that serotonin-induced changes in OCD may be useful for only some components of the disorder; further studies of psychological and biological features that comprise drug-responsive and drug-resistant elements in this disorder are needed. Our previous observations of a lack of any additional benefit from the addition of lithium or triiodothyronine to clomipramine is in contrast to a number of reports indicating that such co-treatment is of benefit to depressed patients. This adds further support to the growing consensus that there are more differences than similarities between depressed patients and OCD patients with secondary depression.

Proposed Course

We are continuing to explore the question of serotonergic factors in OCD and in the mechanism of action of agents that alter OCD symptoms by using 5-HT-selective agonists and antagonists. In addition we plan to study agents acting through other neurotransmitter systems besides serotonin in OCD patients, since it is unlikely that any complex disorder such as OCD is linked solely to a single brain neurotransmitter system dysfunction. We will also study some new rating scales under development to find better symptom and side effect assessment procedures for this patient population. In addition, we are continuing our work concerning the potential of shared neurobiological and clinical features in other neuropsychiatric disorders with compulsive features including anorexia nervosa, bulimia nervosa, trichotillomania and the focal dystonias.

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Pigott, T.A., Grady, T.A., and Rubenstein, C.S.: Obsessive-compulsive disorder and trichotillomania. In: Dunner, D.L. (ed.): Current Psychiatric Therapy W.B. Saunders Co., Inc.

Zohar, J., Insel, T.R., Zohar-Kadouch, R.C., Mueller, E.A., and Murphy, D.L.: Serotonergic role in obsessive-compulsive disorder. In: Belmaker, R.H., (ed.): Progress in Catecholamine Research: Clinical Aspects New York: Alan R. Liss, Inc.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00337-13 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders) Neuropharmacology of Neuroendocrine and Neurotransmitter Regulatory Mechanisms		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Dennis L. Murphy, M.D., Chief, Laboratory of Clinical Science, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Clinical Neuropharmacology		
INSTITUTE AND LOCATION National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: 2.1	PROFESSIONAL: 0.9	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither _(a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p> This project has continued to focus on investigations of the functional status of the <u>serotonin (5-HT)</u> neurotransmitter system in humans using 5-HT-selective agonists (e.g., <u>m-chlorophenylpiperazine</u> (m-CPP), <u>buspirone</u>) and antagonists (e.g., <u>metergoline</u>, <u>ondansetron</u> and <u>trazodone</u>) as pharmacologic probes. A major <u>Pharmacological Reviews</u> article published this year from our group summarized arylpiperazine (including m-CPP)-mediated physiological responses such as blood pressure, neuroendocrine measures, temperature, and subjectively-assessed as well as objectively-rated behavioral changes which have been found to be differentially altered in neuropsychiatric patient subgroups. These alterations in responsivity have been found to differ according to dose, time and route of administration of m-CPP. Additional examples of differential responses to other serotonin agonists and antagonists support the concept that humans possess functionally distinct, independently modulated <u>serotonergic subsystems</u> which appear to correspond, at least in part, to the heterogeneous 5-HT binding sites and neuroanatomical subpathways identified <i>in vitro</i> and <i>in vivo</i> in rodents and some other species. </p>		

Other Collaborative Professional Personnel Engaged on the Project

T. Sunderland, M.D.	Medical Officer	LCS, NIMH
F.M. Jacobsen, M.D.	Guest Scientist	LCS, NIMH
N.A. Garrick, Ph.D.	Guest Researcher	LCS, NIMH
J.L. Hill, Ph.D.	Biostatistician	LCS, NIMH
F. Karoum, Ph.D.	Research Chemist	NB, NIMH
K. Sims, M.D.	Neurologist	E.K. Shriver Ctr., Waltham, MA
X.O. Breakefield, Ph.D.	Geneticist	E.K. Shriver Ctr., Waltham, MA
C. Aulakh, Ph.D.	Senior Staff Fellow	LCS, NIMH
K.P. Lesch, M.D.	Visiting Associate	LCS, NIMH
G. Bagdy	Visiting Fellow	LCS, NIMH
F. Collins	Center for Genetics	John's Hopkins School of Medicine
B.A. Lawlor, M.D.	Assistant Professor	Mt. Sinai Medical School, N.Y.

Project DescriptionObjectives

The principal focus of our work over the last few years has been to develop methodology to evaluate the apparent contributions of changes in brain serotonin (5-HT) function to neuropsychiatric disorders and to the effects of drugs active in treating these disorders. For strategic reasons previously described, we have been attempting to evaluate the state of functional responsiveness (and possibly receptor sensitivity) of the brain 5-HT subsystems using drugs with 5-HT-selective actions as *in vivo* probes of the subsystems.

Most of our investigations in humans are based on previous and ongoing studies exploring the neuroanatomy, physiology, and pharmacology of 5-HT in rodents and other species. Our group continues to use rodent models to test the validity of some of our pharmacologic challenge studies in humans, particularly when more novel agents or dosage regimens are being studied. However, there are some not unexpected neuroanatomical differences in the brain 5-HT systems between primates and rodents and, similarly, species differences have become evident in the rapidly developing area of brain 5-HT receptors and binding sites. Thus, ultimately, 5-HT function needs to be studied directly in humans, particularly when, as some of the preliminary data indicate, neuropsychiatric disorder-specific changes in responsiveness to serotonergic agents occur.

Methods Employed

Human plasma is obtained from blood samples collected via indwelling venous catheters. Plasma from the rodents is obtained by use of indwelling venous catheters that are usually implanted 15 to 24 hours prior to our studies, so that investigations can occur under non-stressful, basal conditions. Some examples of hormones measured by radioimmunoassay include cortisol, prolactin, growth hormone, β -endorphin, melatonin, ACTH, and vasopressin. Serotonin, other monoamines and monoamine metabolites as well as plasma m-CPP concentrations are measured by high performance liquid chromatography with electrochemical detection (HPLC-EC) and by gas chromatography/mass spectrometry. Behavioral changes are assessed using a number of validated self-rating and interviewer's scales.

Major Findings

Most of the clinical studies associated with this project (e.g., those using m-CPP, buspirone and other 5-HT agents) are being carried out in collaboration with other Branches in the NIMH intramural program, and are described in other project reports. A major review of the pharmacological background and current findings from these studies was published this year in Pharmacological Reviews.

In a study of serotonergic modulation of vasopressin, renin and blood pressure, m-CPP was found to elevate plasma vasopressin, mostly likely via an action at 5-HT_{1C} receptors. 5-HT_{1A} agonists were inactive, while increases in renin and in blood pressure appeared to fit a pattern indicating a mediation by 5-HT₂ receptors.

Significance to Biomedical Research and the Program of the Institute

The present results indicate that m-CPP and other agents believed to act through central and/or peripheral serotonergic mechanisms have behavioral, neuroendocrine, cardiovascular, and other physiological effects in humans, which, in general, resemble those seen in other species, especially rodents and nonhuman primates. Limited antagonist studies in humans suggest that many of m-CPP's central effects are most likely to be mediated via 5-HT_{1C} sites although some interactions with 5-HT₂ and possibly 5-HT₃ sites are possible, particularly in the cardiovascular system.

Overall, these studies support the hypothesis that functionally distinct brain 5-HT subsystems exist in humans which correspond, at least in part, to the heterogeneous 5-HT receptors and neuroanatomical subpathways identified *in vitro*. These studies also provide a basis for the comparative evaluation of different physiologic responses which can be used to investigate the status of these different brain 5-HT subsystems in human disorders, as well as changes in these subsystems during psychotherapeutic drug treatment.

Proposed Course

Based on our studies with m-CPP and other 5-HT agonists and antagonists in rodents and monkeys, we are continuing to use these agents as probes to evaluate possible abnormalities in 5-HT function in various neuropsychiatric disorders. Additional studies in patients treated with different classes of antidepressants, anxiolytics and neuroleptics should be important in gaining a better understanding of drug- and disorder-related changes in 5-HT function in humans.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00339-11 LCS
PERIOD COVERED October 1, 1992 to September 30, 1993		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) The Title of The Project Neuropharmacology of Cognition and Mood in Geriatric Neuropsychiatry		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Trey Sunderland, M.D., Chief, Unit on Geriatric Psychopharmacology, LCS, NIMH		
COOPERATING UNITS (if any) Laboratory of Cerebral Metabolism, NIMH; Clinical Brain Disorders Branch, NIMH		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Clinical Neuropharmacology		
INSTITUTE AND LOCATION National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: <div style="text-align: center;">8.5</div>	PROFESSIONAL: <div style="text-align: center;">5</div>	OTHER: <div style="text-align: center;">3.5</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues (c) Neither _(a1) Minors (i) Interviews		
SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided) <p> The Unit on Geriatric Psychopharmacology has focused its efforts on the in depth study of Alzheimer's disease. The scope of investigations range from <u>diagnostic studies</u> to <u>drug trials</u> to <u>basic science laboratory experiments</u>. Diagnostic tests include several newly-created behavioral rating instruments, cerebrospinal fluid investigations, acute drug challenges, neuroendocrine measures, and neuroimaging techniques such as PET and SPECT scans. The drug trials are multifaceted to allow our patients entry to the program at various levels of dementia severity. These drug studies include a short-term trial of DHEA, a long-term trial of deprenyl versus hydergine, and short-term combination trials of physostigmine plus deprenyl, fluoxetine or lithium. Basic science studies focus on the development and characterization of a neuronal cell culture model of Alzheimer's disease using olfactory epithelium from Alzheimer patients and normal controls. These diverse studies are vertically integrated such that any diagnostic finding can be used as a marker for potential therapeutic efficacy and any basic science advance can quickly lead to a potential therapeutic intervention. With this type of coordinated research, the Unit on Geriatric Psychopharmacology has greatly expanded the breadth of its Alzheimer's investigations over the last year. </p>		

Other Collaborative Professional Personnel Engaged on the Project

D.L. Murphy, M.D.	Chief	LCS	NIMH
H. Weingartner, Ph.D.	Guest Researcher	BPB	NIMH
A. Martin, Ph.D.	Special Expert	LCS	NIMH
R. Martinez, M.D.	Guest Researcher	MDARB	NIMH
S.E. Molchan, M.D.	Staff Psychiatrist	LCS	NIMH
B. Vitiello, M.D.	Guest Researcher	BBBS	NIMH
B. Wolozin, M.D., Ph.D.	Staff Fellow	LCS	NIMH
M. Cantillon, M.D.	Staff Fellow	LCS	NIMH
J. Little, M.D.	Staff Fellow	LCS	NIMH

Project DescriptionObjectives

1. Diagnostic Studies in Alzheimer's Disease. Since the diagnosis of Alzheimer's disease remains a problematic clinical issue with a accuracy rate of only 80% until confirmed by autopsy, it would be a major advance to develop a diagnostic instrument or profile for this illness. As a result, we continue to exhaustively characterize our patients from a neuropsychological, biochemical, behavioral, and neuroimaging perspective, both at baseline and longitudinally until death. It is our objective to compare the resultant clinical and biologic profiles of Alzheimer patients with normal controls and other neuropsychiatric populations (i.e., geriatric depression, Parkinson's disease and Korsakoff's disease) and to eventually use these longitudinal profiles as markers for prognostic and therapeutic predictions.

2. Developing and Testing New Pharmacologic Challenge Strategies remains a major research interest of the Unit on Geriatric Psychopharmacology (UGP). This approach is based on the desperate need for new therapeutic approaches with Alzheimer's disease. The Unit has continued its studies of the cognitive and behavioral effects of the anticholinergic agent, scopolamine and has attempted to better understand its anti-memory effects. Because the scopolamine model of memory dysfunction does not completely mimic the memory-deficit of Alzheimer's disease, we are attempting to establish an even better human model. By combining scopolamine with other neurotransmitter selective agents such as metergoline, haloperidol, ondansetron, and mecamylamine, we hope to create a more realistic temporary model of Alzheimer's disease. In so doing, we would ultimately create a human laboratory for the study of memory impairment. Such a development would have important implications for future therapeutic drug development studies as well as the understanding of how human memory works.

3. Combination Treatment Strategies. Since no single agent has been proven effective in the treatment of Alzheimer's disease, we have embarked on a series of studies designed to test for therapeutic synergy among available drugs. This rationale makes a great deal of intuitive sense given the multisystem failure found in the brains of Alzheimer victims and the relative lack of success with the mononeurotransmitter approach of most previous cholinergic trials. Starting with the monoamine oxidase inhibitor, deprenyl, and the cholinergic agent, physostigmine, we are capitalizing on two safe drugs which have both been associated with some modest improvement when used individually. Other combinations of medication will also be used in this treatment strategy, including physostigmine with the serotonin reuptake blocker, fluoxetine.

4. Human Cell Culture Model of Alzheimer's Disease. In a basic science project within the UGP, we are developing a novel human cell culture model for the study of Alzheimer's disease and other neuropsychiatric illnesses. Cultures of proliferating human olfactory neurons have been established using brain tissue obtained at autopsy and under biopsy conditions. Preliminary

studies revealed that these cultured cells have many of the characteristics of central neurons, suggesting that when stressed, they may also express the abnormal biochemical findings of Alzheimer's disease. Immunohistochemical, ultrastructural, and molecular biology studies are currently ongoing with these proliferating cells to explore the exciting possibility that they represent a human *in vitro* model of Alzheimer's disease. Specifically, we are exploring possible abnormalities in the processing of amyloid protein in the Alzheimer cells.

Methods Employed

Diagnostic Criteria. To maximize the accuracy of the clinical evaluation, we test patients with multiple assessment instruments including the Clinical Dementia Rating (CDR) scale of Hughes and coworkers. Strict adherence is paid to the generally accepted DSM-III-R and ADRDA-NINCDS diagnostic criteria. Diagnosis of major affective disorder in elderly subjects is determined using DSM-III-R criteria. Subjects also undergo extensive neuropsychological testing and medical examinations to exclude those with confounding cognitive syndromes or serious medical complications. Autopsy specimens are currently being collected to confirm the diagnosis of Alzheimer's disease whenever possible.

Behavioral and Psychological Assessment. Alzheimer patients frequently do not understand or in some cases misinterpret questions on traditional rating scales of mood and behavior. Consequently, we developed several rating instruments specifically for Alzheimer patients. For example, we created the Dementia Mood Assessment Scale (DMAS) to measure mood changes in these subjects when we found that the standard Hamilton Depression Rating Scale (HDRS) was not appropriate for Alzheimer patients. Ability to perform activities of daily living is also an important measure of functional change in Alzheimer's disease. Once more, we have developed a specific rating instrument [Daily Activities Questionnaire (DAQ)] to begin assessing these skills at baseline and continue longitudinally with input from nursing staff, family, and occupational therapist. Other scales include the modified global (15-point) ratings, Brief Psychiatric Rating Scale (BPRS), and various objective visual analog scales. Elderly depressed subjects are evaluated with more traditional objective and subjective ratings such as the Beck Depression Inventory, HDRS, BPRS, Profile of Mood States, and global rating scales.

Neuropsychological assessment of the geriatric depressed patients and Alzheimer patients involves a large number of standard and experimental testing paradigms. This battery of tests has been extensively updated since the arrival in 1989 of Dr. Alex Martin as a Special Expert in this area. To establish common ground with other research centers studying Alzheimer patients, we test patients with several widely used rating scales such as the Wechsler Memory Quotient, Mini-Mental State Examination, Mattis Dementia Rating Scale, and Boston Naming tests. Patients also undergo exploratory testing of semantic and recognition memory, free recall, vigilance, and attention. Increased emphasis and new experimental paradigms are now being developed for areas of attention, procedural learning, implicit learning, and visual memory, including one test of Clock Drawing which has provided useful clinical and research information.

Biological Assessment. To further exclude other causes of dementia or depression, we obtain multiple biological samples from subjects for research testing. These samples include plasma, platelets, urine, and CSF to measure hormone, monoamine metabolite, and various peptide levels. Various neuroendocrine tests including the TRH stimulation and dexamethasone suppression tests are also employed. Clinical brain imaging techniques including the CT scan, MRI, and SPECT scan are administered. Selected study participants also contribute lymphocytes for further biochemical and genetic analysis.

After the initial baseline evaluation, patients are invited to participate in a series of pharmacologic challenge tests. Patients and normal controls are given intravenous or oral medications (i.e., scopolamine, amphetamine, nicotine, haloperidol, mecamlamine, lorazepam, amphetamine, m-CPP, or TRH) and monitored for several hours following drug administration for physiologic, behavioral, neuroendocrine, and cognitive changes, which are compared to results after placebo administration. The final phase of biological assessment includes short-term and long-term medication trials. Patients can participate in one or more trials that last from eight weeks to two years. During these studies, many of the same biological tests are repeated to help assess the pharmacologic and therapeutic effects of these medications.

Cell Culture Methods

The most exciting methodologic advance in the last year has been our initiation of a human biopsy protocol for the procurement of cell culture material. In collaboration with ENT surgeon Dr. Robert Lebovics (NIDCD), we have developed a safe method to obtain olfactory epithelial tissue from living Alzheimer patients and normal controls. Following a brief surgical procedure, this biopsied material is rapidly transferred to the cell culture facility where we have been successful in establishing propagating cell lines in 10 of our first 11 clinical cases. Those cell lines have the characteristics of neuroblasts, and they along with the previously-obtained autopsy-derived cell lines form the basis for a series of basic science investigations into the pathophysiology of Alzheimer's disease.

Our overall strategy has been first to identify disease related changes and then use these changes to understand the pathophysiology of Alzheimer's disease and, perhaps most importantly, to test for potentially therapeutic agents that can reverse these changes. Human olfactory neuroblasts are grown from olfactory epithelium obtained at autopsy or biopsy. A stable, propagating cell line is generated from each human donor. For investigative studies the cells are grown under basal conditions or in the presence of some specific physiologic stressor, such as the lysosomal blocking agent, chloroquine. The protein or RNA of the cells is then analyzed with specific probes, such as antibodies to amyloid precursor protein (APP), using gel electrophoresis or immunocytochemistry and the amount of the target protein or transcript is quantitated.

Major Findings

1. Diagnostic Studies

Clinically, a major advance has been the publication of the Daily Activities Questionnaire, an objectively-rated functional measures of performance on tasks of daily living. While performance measures are intuitively easy to understand, they are very difficult to measure quantitatively in a reliable fashion, and the DAQ represents several years of successful work in that direction. We have also learned from our retrospective review of over 300 Alzheimer patients and normal controls that there is no association between season birth and Alzheimer's disease.

In our biochemical studies, we have shown that the opiate peptide, dynorphin₁₋₈, is reduced in the cerebrospinal fluid (CSF) of Alzheimer patients versus controls. This work is now being followed up with a collaborative PET neuroimaging study of opiate binding in Alzheimer's disease. We have also shown that contrary to previously published literature, there does not appear to be a difference between Alzheimer's patients and controls in the distribution of lymphocytes in the peripheral blood.

Cognitively, we have published several papers with our neuropsychologist collaborators attempting to further characterize the differences between Alzheimer patients, normal

controls, and other neuropsychiatric populations, including elderly depressed. Specifically, we have shown with Dr. Jordan Grafman that knowledge memory is markedly impaired in Alzheimer's disease as indicated by the reduced ability to complete single scripts. On the other hand, we have documented with Drs. Bruce Pappas and Herb Weingartner that metamemory is much less impaired in Alzheimer patients than episodic memory. With Dr. Alex Martin, we have continued to study the process of semantic priming in Alzheimer's disease, and those projects are still ongoing.

2. Drug Studies

The pharmacologic findings are best divided between our challenge paradigms and the therapeutic studies. In the challenge paradigms, Dr. Susan Molchan has continued our study of the small peptide, thyrotropin releasing hormone (TRH), both in low doses as a neuroendocrine probe and in large doses as a distinct brain active pharmacologic agent. These studies have revealed that TRH partially attenuates the memory impairment caused by scopolamine in young normals but not in older normals, suggesting that the TRH effect is age-related. We have also continued our testing of the scopolamine challenge paradigm and are nearing completion of a large multidrug combination study contrasting the effects of scopolamine with and without other neurotransmitter-selective agents.

From a therapeutic perspective, we have shown this year that selegiline (deprenyl) can safely be coadministered with the cholinergic agent, physostigmine. This is the first step in combination chemotherapy strategy for Alzheimer's disease, and we are already proceeding to study higher doses and other combinations of medications in our patients. Meanwhile, we have documented that the serotonin-selective agent, m-chlorophenylpiperazine (m-CPP), is not of direct short-term benefit to Alzheimer patients. This discovery has helped guide us in the choice of other serotonic agents for our combination studies.

3. Basic Sciences

Our recent focus has been towards lysosomal metabolism. We have made the exciting finding that this pathway of degradation is abnormal in Alzheimer patients. Treatment of Alzheimer neuroblasts with the lysosomal inhibitor chloroquine, elicits an increase in the amount of an 11.5-16 kDa APP degradation products detected that is 7 fold greater in Alzheimer neuroblasts than in neuroblasts from age matched control donors and 14 fold greater than in neuroblasts from young donors ($p < 0.005$). cAMP agonists appear to normalize these abnormalities in APP. Further studies on the metabolism of APP indicate that the degradation of APP varies among cell types, with the two cell types most affected by the illness, neurons and vascular cells, showing the greatest trafficking of APP through the lysosome. This may explain why Alzheimer's disease is an illness of the CNS.

Significance to Biomedical Research and the Program of the Institute

While the development of rating instruments is rarely met with the excitement of a biochemical or genetic finding, objectively measuring functional, behavioral or cognitive change is of paramount importance in Alzheimer research. To this end, the DAQ, an objectively rated measure of functional activities of daily living, is the most recent of our newly-developed clinical measures (following the DMAS for the measurement of depression in dementia and the Clock Drawing Task for the assessment of visuospatial abilities in Alzheimer patients). The DAQ is particularly important because there is no other scale like it which purports to assess function in Alzheimer's disease. Also, the DAQ addresses behaviors across the spectrum of Alzheimer's disease from the early signs, through the middle stages of the illness, until the patient enters the most severe stages of Alzheimer's diseases. As of now, no cognitive, biochemical or other clinical measure promises that kind of breadth of measurement, so we expect the DAQ to be very helpful in quantifying change across our longitudinal studies.

The cholinergic challenge studies represent a continuing attempt to understand the neuropharmacologic basis of learning and memory. While the drug scopolamine alone does not necessarily fully reproduce the memory impairment of Alzheimer's disease, perhaps the combination of scopolamine with other agents such as haloperidol, metergoline, mecamlamine, or ondansetron will better mimic the deficits. Once a better human model of memory impairment is established, then we would have important payoffs. First, the neuropharmacologic underpinnings of memory would be better characterized. Second, we would immediately have a human model of learning in which to test new memory agents (Our experience with TRH blunting the scopolamine effect is an example of this approach). And third, we would have a stronger rationale for the choice of new therapeutic agents with Alzheimer's disease. This last point is particularly important, for it is here that we link the 'pharmacologic challenge strategy' part of our program with the "Combination Therapy Approach." Gains in the challenge strategy should translate quickly into therapeutic recommendations which can subsequently be tested in our clinical populations.

APP would seem to be central to the pathology of AD, yet basic science investigators have been unable to detect abnormal processing of this protein *in vivo* or *in vitro*. Our discovery of abnormal metabolism of APP in olfactory neuroblasts represents the first such demonstration of its kind. This work provides perhaps the first model system for investigating these abnormalities. These findings also demonstrate the value of the olfactory neuroblast cultures in studying neuropsychiatric illnesses. These markers can also serve as *in vitro* markers for potential therapeutic agents.

Proposed Course

We have now carefully characterized over 150 Alzheimer patients and have generated a large clinical, neuropsychologic, and biochemical database on these subjects. With this integrated database, we can perform multilevel analyses of these subjects both at baseline and, more importantly, longitudinally as they progress in their illness. By having spent the time to gather and develop quantitative measures of function, behavior, and diverse cognitive skills, we are in a good position to identify and classify separate groups of our subjects. Alzheimer's disease is most probably a group of disorders or at least a spectrum disorder, and the ability to differentiate subgroups by clinical or even pharmacologic means may well be crucial once better therapeutic options are available.

Pharmacologically the challenge strategies and combination treatment approaches will continue to be a central focus of the UGP. As we learn more about how memory is impaired pharmacologically through our combination challenges (i.e., scopolamine with mecamlamine or scopolamine with ondansetron), we will gain insight into better therapeutic combinations. In this way, the pharmacologic modeling experiments will be closely integrated with the therapeutic protocols. In addition, we will continue to test innovative pharmacologic agents singly (i.e., amphetamine, DHEA, lithium, and fluoxetine) for possible therapeutic effects and to better understand their mechanism of action in elderly subjects. Currently, we are nearing completion of our first chronic therapeutic study of selegiline (deprenyl) and hydergine in Alzheimer's disease, and we are planning further studies of this nature. Which such chronic studies are time-consuming, they must represent a core part of any Alzheimer program.

We are continuing our studies of AD related markers in the olfactory neuroblasts, increasing the number of samples examined, determining the identity of the current markers identified with 2D-gel electrophoresis and expanding the number of markers examined. In this context, we have begun screening cDNA libraries from the neuroblast cell lines. Using the markers currently available, we are surveying chemicals that may have therapeutic value, such as free radical scavengers. Understanding the causes of abnormal metabolism of APP

represents another line of investigation. We are studying the processing of APP, examining lysosomes from AD donors and are using recombinant forms of APP to identify mechanisms of amyloidogenesis, *in vitro*. In addition, we are obtaining APP constructs harboring the mutant forms of APP for use in transfection studies. Finally, we are forwarding our studies understanding the regulation of differentiation of this novel cell culture system.

Now that PET and SPECT imaging has progressed beyond the measurement of metabolism and/or blood flow, we are putting a great deal of effort into brain imaging studies over the next several years. In collaboration with Robert Cohen, M.D., Ph.D. (PET) and Daniel Weinberger, M.D. (SPECT), we have the opportunity to test relatively specific ligands in patient groups versus controls and we have already initiated several studies of the opiate and cholinergic systems, respectively. As one example, we are testing the "upregulation plasticity" of the cholinergic system in humans as measured by QNB-SPECT following chronic treatment with cholinergic agents. While this may at first glance seem to be yet another imaging study, it actually combines neurotransmitter-specific SPECT technology and a specific psychopharmacologic treatment with the beauty of a before and after within-subjects research design. If successful, we will have used a powerful neuroimaging tool to transform what have previously been limited to "in vitro" animal research projects to studies of "in vivo" neuronal plasticity in living human beings, (i.e., functionally mapping important neurotransmitter receptor changes associated with aging, dementia, and drug treatment). This line of clinical research could represent a major advance for both the study of neuropsychiatric disease as well as the developing field of neuroimaging.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02588-02 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Cognitive Dysfunction in Dementia and Related Neuropsychiatric Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Alex Martin, Ph.D., Chief, Cognitive Studies Unit, LCS, NIMH		
COOPERATING UNITS (if any) Henry M. Jackson Foundation for the Advancement of Military Medicine, Rockville, MD Uniformed Services University of the Health Sciences, Bethesda, MD		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Clinical Neuropharmacology		
INSTITUTE AND LOCATION National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892		
TOTAL STAFF YEARS: <div style="text-align: center;">6</div>	PROFESSIONAL: <div style="text-align: center;">3</div>	OTHER: <div style="text-align: center;">3</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <p>Our research program has two main components. Firstly, to develop and test models of cognitive processes via study of the way these processes breakdown following brain injury or disease. Secondly, to evaluate the cognitive status of psychiatric patients in order to test hypotheses concerning possible neuroanatomic correlates of these disorders. Our studies have utilized priming paradigms that place minimal demands on attentional and retrieval processes to assess the integrity of <u>object recognition, naming, visuospatial processes and memory</u> in patients with <u>Alzheimer's disease</u>. We have obtained evidence in support of a model that posits that <u>posterior cortical pathology</u> results in a selective degradation of previously acquired knowledge, rather than an inability to retrieve information from intact knowledge stores. These degraded knowledge representations are, in turn, proposed to be responsible for impaired naming and to substantially contribute to poor memory in patients with Alzheimer's disease.</p> <p>Our studies of individuals infected with the <u>Human Immunodeficiency Virus</u> (HIV) have found slowed response times, impaired motor-skill learning and other subtle deficits consistent with involvement of subcortical regions of the brain in a subgroup of subjects. The significant relationship between task performance and concentrations of a potent neurotoxin, <u>quinolinic acid</u>, in the cerebral spinal fluid of the HIV+ subjects, suggested that the deficits may be primarily due to infection of the CNS. In contrast, adults with <u>Obsessive Compulsive Disorders</u> (OCD) performed normally on similar tasks suggesting that the types of deficits seen in patients with structural lesions of the basal ganglia and other subcortical regions may provide a good model for understanding HIV-related cognitive dysfunction, but not for adult OCD.</p>		

Other Collaborative Professional Personnel Engaged on the Project

D. L. Murphy, M.D.	Chief	LCS	NIMH
T. Sunderland, M.D.	Staff Psychiatrist	LCS	NIMH
F. M. Lalonde, Ph.D.	Research Psychologist	LCS	NIMH
C. L. Wiggs, Ph.D.	IRTA, Research Psychologist	LCS	NIMH
M. P. Heyes, Ph.D.	Visiting Scientist	LCS	NIMH
S. P. Markey, Ph.D.	Pharmacologist	LCS	NIMH
M. Alterman, M.D.	Staff Psychiatrist	LCS	NIMH
A. M. Salazar, M.D.	Professor of Neurology	USUHS	
W. A. Law, Ph.D.	Research Psychologist	HMJF	
R. L. Mapou, Ph.D.	Research Psychologist	HMJF	

Project DescriptionObjectives, Methods and Major Findings.

1. Knowledge Representations in Patients with Alzheimer's Disease. Difficulty naming objects and other word-finding deficits are common and severely disabling problems for patients with Alzheimer's disease (AD) and other individuals with disease or injury to specific regions of the left hemisphere. Based on a variety of evidence we proposed that word-finding problems in AD patients were due to an actual deterioration of semantic knowledge, rather than a failure to retrieve information from an intact knowledge store as others have argued. Specifically, we maintained that, as a result of neuropathology in the posterior regions of the left temporal lobe, semantic representations become over-generalized due to a loss or dysfunction of neurons responsible for the coding of object-specific attributes and features. A central and direct prediction of this model is that AD patients should show abnormally large facilitation of reaction time (RT), or hyperpriming, when they are required to make a semantic judgement about an object that has been immediately preceded by a semantically-related object. Using a newly developed task, we found that the presentation of a picture of an object for a very short duration (150 msec) that is then followed, after a short interval (500-1000 msec), by a semantically-related object, will produce a significant reduction of response time for both AD patients and normal controls relative to when there is no semantic relationship between the two pictures. More importantly, as predicted by our model this semantic priming effect was of proportionally greater magnitude for the AD patients than it was for healthy, age-matched control subjects. Additional studies with normal elderly subjects using physically degraded pictures have demonstrated that the increased facilitation observed in AD is not due to generalized slowing of RT. These findings provide support for the hypothesis of degraded semantic representations and suggests that object naming problems in patients with AD, and perhaps other patients with naming problems that result from posterior left temporal lobe damage, may be due to an actual degradation of object representations in the brain.

In addition to having difficulty naming objects and performing other tasks dependent on knowledge acquired prior to the onset of disease, AD patients have severe difficulty acquiring new knowledge. Recent evidence suggests that patients who are amnesic as a result of isolated damage to the medial temporal region can learn some types of information. For example, learning can be demonstrated in severely amnesic patients when they are tested under conditions that do not require explicit recall or recognition of previously presented material (implicit learning). The limits of this type of learning, especially in patients with combined lesions of both medial temporal region and cortical zones, as is characteristic of the brains of AD patients, has not been adequately tested.

In a series of studies we have shown that whether or not implicit memory is preserved in AD patients is dependent on the way learning is measured, and on the type of material they are required to retain. Unlike amnesic patients with damage limited to the medial temporal lobe, the AD patients did not show implicit learning when measured by facilitation of the ability to name previously studied objects. These results suggested that this type of implicit learning is dependent on an intact cortex. However, normal implicit memory was observed when learning was assessed by facilitation of the speed to which semantic judgements could be made about the previously seen objects, even though the AD patients' explicit recognition of these objects was extremely poor. These data suggest that even in individuals with relatively wide-spread cortical pathology, some form of representation can be created in response to recently presented material and that this representation or trace can be re-activated at a latter time. In marked contrast, no evidence of implicit learning was found for either AD patients, normally elderly or young control subjects when novel, unstructured spatial information was substituted for the objects, even though the normal subjects could explicitly remember the material. These results suggest that implicit learning is dependent on the activation of previously acquired representations or on the use of material that is amenable to processing by these representational systems.

2. Subtle Motor and Cognitive Impairments in HIV-infected Individuals. In collaboration with colleagues at the Uniformed Services University of the Health Sciences, Walter Reed Army Medical Center, and the Henry M. Jackson Foundation for the Advancement of Military Medicine we have been engaged in a longitudinal study of subjects in the relatively early stages of HIV-1 infection as defined by the Walter Reed staging system. We have found that although these subjects performed normally on many standard neuropsychological tests, a subset of individuals (20-30%) had slowed reaction time (RT), impaired motor-skill learning, and deficits on other tasks sensitive to subcortical dysfunction. These subtle impairments were found in comparison to control groups composed of HIV-seronegative normal individuals, and seronegative psychiatric patients matched with the HIV+ subjects on degree of self-reported anxiety and depression.

Concurrently, in collaboration with Drs. Melvyn Heyes and Sanford Markey of the LCS Section on Analytical Biochemistry, we have found significantly elevated concentrations of an endogenous neurotoxin, quinolinic acid (QUIN), in the cerebral spinal fluid (CSF) in a some of these subjects. This excitotoxin is an agonist of the N-methyl-D-aspartate (NMDA) receptor which are highly concentrated in select regions of the brain; especially the hippocampus and basal ganglia. CSF QUIN increased over time and was significantly correlated with RT and motor-skill learning. In contrast, no significant relationships were observed between neuropsychological tasks and measures of immunological status (CD4 or CD8 cell counts).

Our findings suggest that subtle, but statistically significant neuropsychological impairment can occur during the relatively early stages of infection in at least some individuals. It is important to stress, however, that the majority of subjects were, and remain, unimpaired, and that the observed deficits were relatively subtle in nature. This last point suggests that these impairments may not interfere with real-world performance, but this issue has yet be explored. It should also be noted that the relationship between these subtle deficits and the development of overt dementia remains to be elucidated. Our studies also suggest that reaction time tasks may be the most sensitive behavioral indicator of CNS dysfunction and thus may prove to be useful for quantifying the presence and progression of HIV-related CNS disease, and that quinolinic acid may play an important role in the development of cognitive impairment in HIV+ individuals.

3. Cognitive Processing in Adults with Obsessive Compulsive Disorders. It has recently been proposed that the basal ganglia region may be the primary site of brain dysfunction in patients

suffering from Obsessive Compulsive Disorders (OCD). The objective of our study was to test this hypothesis by using cognitive measures previously shown to be sensitive to damage to these brain regions (eg., patients with Huntington's disease).

Contrary to expectation, neither unmedicated OCD patients nor patients with a related anxiety disorder (trichotillomania) were impaired on any of the cognitive tasks, including RT measures of parallel and serial visual processing, egocentric spatial ability, and motor-skill learning. These data suggest that the type of cognitive and motor deficits often found in patients with basal ganglia lesions are not likely to provide a useful model for the neuropsychology of obsessive compulsive disorders.

Significance to Biomedical Research and the Program of the Institute

During the past decade, considerable gains have been achieved in our ability to detect specific types of cognitive deficits. However, our understanding of the nature of these impairments has remained largely incomplete. For example, naming difficulties are a relatively common sequela of a variety of different disorders and can occur following damage to widely disparate areas of the brain. Our ability to develop cognitive probes that will allow us to distinguish between deficits due to impaired attention, retrieval mechanisms, degraded storage and the like will advance our understanding of the underlying architecture or organization of specific brain systems. Such knowledge will also provide a theoretical framework for the design of rationale, cognitive remedial procedures and techniques.

The development and refinement of new testing procedures and their validation on patients with verified brain damage will provide new tools for exploring the relationship between cognitive dysfunction and psychiatric disorders of mood and thought. Our studies of OCD patients, for example, highlight the rather remarkable extent to which cognition and mood can be dissociated. Our studies of HIV-infected individuals have also suggested that self-reported mood changes have a negligible role on neuropsychological test performance. Nevertheless, using tasks that were specifically chosen because of their sensitivity to subcortical involvement, we found subtle changes in some HIV+ individuals prior to the on-set of clinically-apparent symptoms. The relationship between performance on these tasks and cerebral spinal fluid concentrations of quinolinic acid have strengthened the possibility that these changes may be primarily due to nervous system dysfunction. Given the large number of individuals currently estimated to be infected with HIV, our ability to provide accurate early detection of central nervous system involvement will become of increasing importance for therapeutic intervention.

Proposed Course

We will continue to explore the nature of object representations, spatial processing, and other types of knowledge systems in patients with Alzheimer's disease. An important component of our future studies will be the inclusion of patients with other types of dementias, especially those with primarily subcortical/frontal lobe pathology (eg., Huntington's, Parkinson's). These patient groups will be compared on tasks designed to distinguish degraded store disorders, as seen in Alzheimer's patients, from the type of retrieval-based deficit believed to underlie impairments in the so-called "subcortical" dementias. Studies using positron emission tomography to explore the neuroanatomical correlates of object recognition, perceptual closure, object naming and memory processes in both normal subjects and Alzheimer's patients will also be pursued.

Based on our preliminary findings with HIV-infected subjects, a collaborative, multi-center study of Army, Navy, and Air Force HIV-infected personnel has been instituted using a battery of tests based on the NIMH Neuropsychological Battery for the assessment of HIV-related

cognitive change. This study should provide critical information concerning the nature and prevalence of HIV-related changes in cognition and mood. We will also continue our longitudinal evaluation of the impaired subjects identified in our earlier studies to determine whether slowing of reaction time and poor motor-skill learning are predictive of the development of dementia during the latter stages of illness. Finally, continued study of OCD patients focusing on the relationship between mood, attention and memory are in-progress.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES • PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00433-12 LCS
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT Role of neuropeptides and biogenic amines in neuroendocrine regulation		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, M.D., laboratory, and institute affiliation) PI J.M. Saavedra, Chief, SP, LCS, NIMH Others: K. Tsutsumi, Visiting Scientist, SP, LCS, NIMH M. Viswanathan, Visiting Associate, SP, LCS, NIMH A. Seltzer, Visiting Associate, SP, LCS, NIMH C. Stromberg, Visiting Fellow, SP, LCS, NIMH S. Zorad, Visiting Fellow, SP, LCS, NIMH L. Naveri, Visiting Fellow, SP, LCS, NIMH		
COOPERATING UNITS (if any) Dept. Neurology, USUHS. Lab. Molec. and Integrative Neurosc., NIEHS. Lab. Cell. Development & Oncology, NIDR. Dept. Physiol., Univ. Florida at Gainesville. Dept. Histology & Neurosc., Karolinska Inst., Finland		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Pharmacology		
INSTITUTE AND LOCATION NIMH-NIH, Bethesda, MD		
TOTAL STAFF YEARS: 10	PROFESSIONAL: 9	OTHER: 1
CHECK APPROPRIATE BOX(ES) _ (a) Human subjects _ (b) Human tissues <u>X</u> (c) Neither _ (a1) Minors (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) <u>Angiotensin II receptors:</u> <p>In the rat brain, the <u>angiotensin II receptor subtype AT₁</u> is heterogeneous and has been subclassified as <u>AT_{2A}</u> (sensitive to <u>guanine nucleotides</u> and <u>DTT</u>) and <u>AT_{2B}</u> (insensitive to guanine nucleotides and DTT). Each subgroup has a specific localization in brain.</p> <p><u>AT₁</u> and <u>AT_{2A}</u> and <u>AT_{2B}</u> receptors are selectively localized in the rat <u>fetus</u> and their characteristics are similar to those of the adult. Novel sites of localization for <u>AT_{2B}</u> receptors have been found in the <u>deep cerebellar nuclei</u>, the <u>superior sagittal sinus</u> and the <u>cranial nerve nuclei</u>.</p> <p>Both subtypes, <u>AT₁</u> and <u>AT₂</u>, <u>angiotensin II receptors</u>, play a role in <u>cerebral blood flow autoregulation</u>.</p> <p>In the rat brain, the <u>median eminence</u> contains exclusively <u>AT₁</u> receptors. These receptors are coupled to <u>PI turnover</u>, but not to the <u>cAMP</u> or <u>cGMP</u>.</p> <p>At a certain age (1 week), the expression of <u>aortic AT₁ receptors</u> is enhanced in <u>spontaneously hypertensive rats</u>.</p> <p>In the rat <u>spleen</u>, <u>AT₁</u> receptors are coupled to <u>PI turnover</u>.</p> <p>The <u>adult kidney</u> expressed only <u>AT₁</u> receptors. Conversely, the <u>fetal rat kidney</u> expressed mainly <u>AT₂</u> receptors.</p> <p>High expression of <u>AT₂</u> receptors occurs <u>experimental wound healing</u> in subcutaneous tissues.</p> <p>High expression of <u>AT₁</u> receptors occurs in the <u>aortic neointimal smooth muscle cells</u> formed after <u>balloon angioplasty</u>.</p> <p>In vivo administration of the <u>AT₁ antagonist losartan</u> blunts the secretion of <u>ACTH</u> during <u>acute immobilization stress</u>.</p>		

Addition to PRINCIPAL INVESTIGATOR

F. Heemskerk,	Visiting Fellow, SP, LCS, NIMH
M. Torres,	Guest Researcher, SP, LCS, NIMH
K. Michels,	Guest Researcher, SP, LCS, NIMH
G. Ciuffo,	Guest Researcher, SP, LCS, NIMH
F. Correa,	Guest Researcher, SP, LCS, NIMH

Addition to COOPERATING UNITS

Center for Molecular Medicine, Berlin, Germany.
 Dept. Biochem., Vanderbilt Univ. School of Medicine.
 Dept. Cell Biol. and Anatomy., The University of Alabama.

PROJECT DESCRIPTION

Objectives

To study the role of angiotensin II in the modulation of cerebrovascular autoregulation, pituitary function, immune system and autonomic nervous system activity.

To study the role of angiotensin II in growth and development.

To study the role of angiotensin II receptor subtypes in the regulation of cerebral blood flow.

To study the signal transduction mechanisms and functions of selective angiotensin receptor ligands.

Methods employed

Neuroanatomical, surgical, biochemical (RIA, gel electrophoresis, radioenzymatic assays, insitu hybridization, high pressure liquid chromatography), autoradiography with image analysis combined with computerized microdensitometry, in vivo determination of cerebral blood flow (Laser-doppler-flowmetry), and use of genetic and experimental animal models of disease.

Major findings

1. Angiotensin II receptor subtypes (AT₁ and AT₂) participate in the modulation of cerebrovascular autoregulation in the rat. Both the selective AT₁ antagonist Losartan, and the selective AT₂ displacer PD 123319 extend the upper limit of cerebrovascular autoregulation towards higher blood pressures.
2. The ratio of AT₁/AT₂ receptors depends on the developmental stage, both in the brain and in peripheral tissues. Higher expression of AT₂ receptors occurs early during development.
3. The expression of AT₁ and AT₂ receptors is also changed as a response to injury. AT₁ receptor number is increased in the newly formed aortic neointima after endothelium denudation. AT₂ receptor number increases during experimental wound healing, and after tumor growth.
4. Administration of the selective AT₁ antagonist losartan diminishes the hypothalamic-pituitary-adrenal response to acute stress.

Significance to Biomedical Research and to the Institute

Our studies indicate that angiotensin II plays a role in the regulation of cerebral blood flow. Pharmacological modification of selective subtypes of angiotensin II receptors could advance our understanding of the mechanisms of cerebrovascular function, of the mechanisms of central action of newly developed antihypertensive drugs, and could prove useful for the treatment or prevention of stroke and other cerebrovascular disorders.

Our studies support the hypothesis of a role for AT₂ receptors during development and organogenesis. In the brain, AT₂ receptors could be related to the development of sensory and motor systems. In vascular and other peripheral tissues, both AT₂ and AT₁ receptors could be involved in the process of tissue repair and growth.

Angiotensin II is an important stress hormone, and selective blockade of angiotensin II receptors could result in beneficial effects during stress.

The knowledge of the biochemical characteristics of angiotensin II receptors could be useful to better understand their biological roles and mechanisms of action.

Our studies may have relevance for the treatment of conditions involving alterations of cardiovascular and cerebrovascular function, growth and tissue repair, and stress.

Proposed course

We plan to further advance our studies along the following lines:

1. The study of the influence of angiotensin II and selective receptor agonists/antagonists on cerebrovascular control. We will study the influence of these compounds on cerebrovascular autoregulation, cerebral vasospasm, and cerebral hemorrhage. We hope to find pharmacological means for the prevention and treatment of cerebrovascular disease.
3. We will continue to study the role of angiotensin receptor subtypes in the mechanisms of growth and repair, to elucidate their role as vascular and tissue growth factors.
4. We will use animal models of neurological mutants to further explore the role of AT₂ receptors on the development and organogenesis of central sensory and motor systems.
5. We will use molecular biology techniques to study the regulation of the formation, expression and metabolism of selective angiotensin II receptor subtypes.
6. We will continue to attempt to elucidate the presently unknown second messenger systems for AT₂ receptors, and we will attempt to purify AT₂ receptors, to further elucidate their biochemical characteristics.

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IN PRESS

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Saavedra JM, Zorad S, Tsutsumi K. Localization of atrial natriuretic peptide B and C receptors in rat brain. Handbook of Chemical Neuroanatomy, Neuropeptide receptors in the CNS, Part III. M. Kuhar and T. Hökfelt (eds). Elsevier Science Publishers Biomedical Division, Amsterdam, The Netherlands.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00796-07 LCS
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Cytochemical Tracing of Thalamic Connections with Midline Frontal Cortex		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	P.D. MacLean	Intramural Research Scientist LNP, NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science, NIMH		
SECTION Section on Comparative Studies of Brain and Behavior		
INSTITUTE AND LOCATION NIMH, NIH, Poolesville, Maryland 20837		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) _ (a) Human subjects _ (b) Human tissues _ (c) Neither _ (a1) Minors _ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided) Work on this project transferred to project Z01 MH 00851-2 8 LNP.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 00797-07 LCS
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Neurobiology of Attachment		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> P.I. T.R. Insel Staff Physician LNP, NIMH </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science, NIMH		
SECTION Section on Comparative Studies of Brain and Behavior		
INSTITUTE AND LOCATION NIMH, NIH, Poolesville, Maryland 20837		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither </div> <div style="display: flex; margin-top: 5px;"> <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided) Work on this project was transferred to project ZO1 MH 01104-01 LNP.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 00799-06 LCS
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Studies on Postnatal Neuronogenesis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	B. B. Stanfield	Research Biologist LNP, NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science, NIMH		
SECTION Section on Comparative Studies of Brain and Behavior		
INSTITUTE AND LOCATION NIMH, NIH, Poolesville, Maryland 20837		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) _ (a) Human subjects _ (b) Human tissues _ (c) Neither _ (a1) Minors _ (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided)		
Work from this project was transferred to project ZO1 MH 01105-01 LNP.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02219-09 LCS
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders) Animal Models of Anxiety		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> P.I. T.R. Insel Staff Physician LNP, NIMH </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science, NIMH		
SECTION Section on Comparative Studies of Brain and Behavior		
INSTITUTE AND LOCATION NIMH, NIH, Poolesville, Maryland 20837		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither </div> <div style="margin-left: 20px;"> <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div>		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided) Work from this project was transferred to project Z01 MH 01104-01 LNP.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02482-04 LCS
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Titles must fit on one line between the borders) Comparative Cytoarchitecture of the Cingulate Cortex		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
P.I.	P.D. MacLean	Intramural Research Scientist LNP, NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science, NIMH		
SECTION Section on Comparative Studies of Brain and Behavior		
INSTITUTE AND LOCATION NIMH, NIH, Poolesville, Maryland 20837		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) _ (a) Human subjects _ (b) Human tissues _ (c) Neither _ (a1) Minors _ (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided)		
Work on this project transferred to project ZO1 MH 00851-28 LNP.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00382-18 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) LOCALIZATION AND CHARACTERIZATION OF BRAIN NEUROCHEMICALS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
Dr. Lois Winsky	Staff Fellow	LCS, NIMH
Dr. David M. Jacobowitz	Chief, Section on Histopharmacology	LCS, NIMH
Dr. Tomoko Yamaguchi	Guest Worker	LCS, NIMH
Dr. Brian Martin	Senior Staff	NSB, NIMH
Dr. Pascale Montpied	Staff Fellow	NSB, NIMH
Dr. Robert Wenthold	Senior Staff	NDI, NIH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Histopharmacology		
INSTITUTE AND LOCATION National Institute of Mental Health, Building 10, Room 3D-48, Bethesda, Maryland		
TOTAL MAN-YEARS: 1.8	PROFESSIONAL: 1.4	OTHER: .4
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A detailed mapping of <u>calretinin</u> positive cells and fibers in the rat <u>thalamus</u> was completed using <u>in situ</u> hybridization histochemistry and immunocytochemistry. Results of this study revealed populations of calretinin positive cells in several discrete thalamic nuclei (e.g., reticular, rhomboid, reuniens, paraventricular) and in regions which overlapped defined nuclear thalamic boundaries (e.g., cells in the central and intralaminar nuclei continuous with the central grey).</p> <p>Unilateral cochlea ablations were found to increase the immunoreactivity of neurons in the ipsilateral ventral cochlear nucleus and contralateral trapezoid body. However, no changes were seen in mRNA label.</p> <p><u>In situ</u> hybridization histochemistry revealed that calretinin mRNA is present early in development (E9) in chick embryos.</p> <p>A 39 kDa protein, whose phosphorylation is inhibited by calretinin, was found predominantly in <u>mitochondrial membranes</u> and was localized in several peripheral tissues with greatest amounts in the testis. Calretinin inhibited the phosphorylation of this protein in all regions and subcellular fractions where the 39 kDa band was visible on autoradiograms. Divalent cations stimulated both the phosphorylation of the 39 kDa (Mg^{2+}, Mn^{2+}, Ni^{2+}, Ca^{2+} or Co^{2+}) and the inhibition by calretinin (Mg^{2+}, Co^{2+} or Ni^{2+}). Zn^{2+} inhibited both the phosphorylation of the 39 kDa band and the effect on calretinin, while EDTA attenuated the calretinin effect. Calretinin also produced a slight attenuation in the phosphorylation of a 44 kDa band identified as the alpha subunit of pyruvate dehydrogenase. In contrast, initial examination of the effects of a related calcium binding protein (calbindin D-28k) revealed a stimulation of protein phosphorylation and a reversal of the inhibitory effect of calretinin on phosphorylation of the 39 kDa protein.</p>		

PROJECT DESCRIPTION:

Objectives: (1) To continue the descriptive studies of calretinin localization within brain; (2) To examine the effects of calretinin and related proteins on phosphorylation systems in brain; (3) To further characterize the 39 kDa protein and ultimately determine the identity of this protein.

Methods Employed: (1) Immunocytochemical methods; (2) *In situ* hybridization histochemistry; (3) Subcellular fractionation by sucrose density centrifugation; (4) Protein phosphorylation; (5) One and two-dimensional gels; (6) Protein blotting onto nitrocellulose membranes; (7) Liquid scintillation counting of radiolabeled proteins cut from gels.

Major Findings:

- (A) The combined *in situ* hybridization and immunohistochemical mapping of the rat thalamus revealed a close matching of calretinin positive cells by both methods. Results of this study highlighted several thalamic nuclei which contained abundant calretinin including the paraventricular, reticular, rhomboid, reuniens, lateroposterior medial rostral, lateral geniculate and subparafascicular nuclei. No positive cells were found in the anterior, ventral or posterior nuclei. These results revealed calretinin as a unique identifying marker for distinct sets of thalamic neurons.
- (B) An examination of the effect of calretinin on protein phosphorylation in subcellular fractions identified a 39 kDa protein band as the major substrate whose phosphorylation is modified by calretinin. This 39 kDa band was most prominent in mitochondrial membranes of brain and several peripheral tissues. Incorporation of radiolabeled phosphate into the 39 kDa band was greatest in the testis and lowest in the liver. Intermediate levels of phosphorylation were observed in brain, spleen, kidney, heart and lung. Calretinin inhibited the phosphorylation of this protein in all regions and subcellular fractions where the 39 kDa band was visible on autoradiograms.
- (C) Calretinin produced a dose dependant reduction in the phosphorylation of the 39 kDa band with decreases of 25% and 53% at 0.1 and 1 μ M, respectively. Examination of the time course of effects revealed that the 39 kDa band was maximally phosphorylated within 15 sec, while

the inhibition by calretinin was most apparent after 2 min. These effects were pH dependant with maximal inhibition of phosphorylation by calretinin at pH near neutrality. Phosphorylation of the 39 kDa band was observed in the presence of 5 mM EDTA while the effect of calretinin was inhibited under this condition. The inhibitory effect of calretinin was enhanced in the presence of Mg^{2+} , Co^{2+} and Ni^{2+} while the basal phosphorylation of the 39 kDa was stimulated by Mg^{2+} , Mn^{2+} , Ni^{2+} , Ca^{2+} and Co^{2+} . Zn^{2+} inhibited both the phosphorylation of the 39 kDa band and the effect of calretinin. Calretinin also attenuated the phosphorylation of a 44 kDa band. Based on several studies (co-migration with purified enzyme, immunoblots, response to effectors of the pyruvate kinase), it was concluded that this 44 kDa phosphoprotein was the alpha subunit of pyruvate dehydrogenase.

- (D) Calbindin D-28k, a calcium binding protein closely related to calretinin, enhanced the phosphorylation of several proteins in both mitochondrial and synaptic membrane fractions of rat brain. Calbindin D-28k (0.1 μM) inhibited the effect of calretinin (0.2 μM) on the phosphorylation of the 39 kDa band.
- (E) Examination of the auditory nuclei of guinea pigs one week after unilateral destruction of the cochlea revealed a significant increase in the immunohistochemical label of cells in the ipsilateral ventral cochlear nucleus and the contralateral trapezoid body. However, the lesion did not appear to effect calretinin mRNA as no differences were found in the *in situ* hybridization histochemical label in these or other nuclei.
- (F) Examination of the calretinin mRNA label in embryonic chicks (using *in situ* histochemistry) revealed expression of calretinin mRNA occurs early in development and is present at E9.

Significance to Biomedical Research and the Program of the Institute:

The thalamus is known to act as a gatekeeper, integrating messages from lower brainstem regions and regulating the activation of the cerebral cortex. It has been implicated in the control of numerous functions such as sleep, learning and memory, and movement behavior. In addition, the thalamus has been implicated in the etiology of some diseases (e.g., epilepsy). The mapping of calretinin cells and fiber connections provides a new chemotectonic categorization of thalamic neurons

which will prove useful in future studies of thalamic function and the role of calretinin in thalamic cells. The phosphorylation studies of calretinin are the first to examine function of this neuronal-specific calcium binding protein. The finding that the 39 kDa protein is isocitrate dehydrogenase suggests a possible functional role of calretinin in modifying mitochondrial enzyme activity. In addition, studies of calretinin interaction with mitochondrial enzymes could provide clues towards understanding some neuronal defects known or hypothesized to be the result of metabolic disorders.

Proposed Course of the Project:

To isolate and purify enough of the 39 kDa protein in order to obtain a partial amino acid sequence for positive identification and for the production of a polyclonal antibody. This antibody will be used to screen a cDNA library in order to eventually clone the gene. In addition, assays will examine the effect of calretinin on several mitochondrial enzymes. We will also examine the effects of calbindin D-28kDa, a closely related calcium binding protein, on protein phosphorylation in various subcellular fractions and animal tissues. To continue studies of the effects of monaural cochlea destruction on calretinin mRNA and immunohistochemical label to include earlier survival times. To examine the development of calretinin positive cells and the effects of prenatal unilateral cochlea removal on the developing chick embryo.

Publications

Arai, R., Winsky, L., Arai, M. and Jacobowitz, D.M.: Immunohistochemical localization of calretinin in the rat hindbrain. J. Comp. Neurol. 310:21-44, 1991.

Winsky, L. and Jacobowitz, D.M.: Radioimmunoassay of calretinin in rat brain. Neurochem. Internat. 19:517-552, 1991.

Dechesne, C.J., Winsky, L., Kim, H.N., Goping, G., Vu, T.D., Wenthold, R.J. and Jacobowitz, D.M.: Identification and ultrastructural localization of a calretinin-like calcium binding protein (protein 10). Brain Res. 560:139-148, 1991.

Yamaguchi, T., Winsky, L. and Jacobowitz, D.M.: Calretinin, a neuronal calcium binding protein inhibits phosphorylation of a 39 kDa synaptic membrane protein. Neuroscience Lett. 131:79-82, 1991.

Winsky, L., Montpied, P., Arai, R., Martin, B.M. and Jacobowitz, D.M.: Calretinin distribution in the thalamus of the rat: Immunohistochemical and *in situ* hybridization histochemical analyses. Neuroscience 50:181-196, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-MH 00388-16 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Coexistence of Peptides and Neurotransmitters		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) Dr. David M. Jacobowitz Chief, Section on Histopharmacology LCS, NIMH		
COOPERATING UNITS (if any) Dr. Tamas F. Freund Department of Functional Neuroanatomy, Hungarian Academy of Sciences Budapest, Hungary		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20892 - Building 10, Room 3D-48		
TOTAL MAN-YEARS: .1	PROFESSIONAL: .1	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The possible coexistence of calretinin with other <u>calcium binding proteins, parvalbumin and calbindin D28k</u>, and with <u>GABA</u>, was studied in non-pyramidal cells of the rat dorsal <u>hippocampal formation</u>. The majority of the calretinin-containing neurons (83%) were found to be immunoreactive for GABA (79%) in the dentate gyrus, 84% in the CA2-3, and 88% in the CA1 subfield). Analysis of the calretinin-immunoreactive cells of these subfields revealed that the two morphologically distinct types of calretinin neurons, i.e., the spiny and the spine-free cells, differ in their immunoreactivity for GABA. The overwhelming majority (92%) of the spine-free neurons were GABA-positive, whereas the immunoreactivity of spiny cells was ambiguous. At the sensitivity threshold of the immunocytochemical techniques used in the present study, most of the spiny cells (89%) had to be considered as GABA-negative. Colchicine treatment resulted in a degeneration of calretinin-immunoreactive neurons; therefore, its effect on the GABA content of spiny neurons could not be evaluated. Nevertheless, the observations suggest that calretinin-containing neurons are heterogeneous both morphologically and neurochemically. Examination of the coexistence of calcium binding proteins revealed that none of the hippocampal cells contained both calretinin and parvalbumin in any regions of the hippocampus. Some overlap was detected between the calretinin- and the calbindin-containing cell populations, 5.1% of the former and 6.2% of the latter were immunoreactive for both calcium binding proteins. This may be due to a small degree of cross reactivity with calretinin. Thus, these results demonstrate that the majority of calretinin-immunoreactive neurons are GABAergic and represent a subpopulation of non-pyramidal cells with no or only a negligible overlap with the subpopulations containing the other calcium binding proteins, parvalbumin and calbindin. </p>		

PROJECT DESCRIPTION:

Objectives: To determine whether the subpopulations of non-pyramidal cells of the hippocampus containing the 3 different calcium binding proteins (calretinin, calbindin, parvalbumin) show any overlap, and whether the spiny and spine-free calretinin-IR neurons were also immunoreactive for GABA.

Methods Employed: A peroxidase immunocytochemical method.

Major Findings:

- (A) The majority of calretinin-IR neurons are immunoreactive for GABA.
- (B) The two types of calretinin-IR neurons were different in their GABA content, the majority of spine-free cells were found to be GABA-positive, whereas only a small number of the spiny calretinin neurons were unequivocally immunoreactive for GABA.
- (C) Calretinin- and parvalbumin immunoreactivity was never found in the same neurons, whereas some overlap (approximately 5%) was observed between the calretinin- and calbindin D28k-containing subpopulations of interneurons.
- (D) Only two calretinin-IR neurons in the hilus were found to project commissurally.

Significance to Biomedical Research and the Program of the Institute:

The conclusion can be drawn that calcium binding proteins are important markers for the functional classification of different groups of inhibitory interneurons even though the role of calcium binding proteins themselves is not fully understood. Calcium binding proteins have recently attracted interest since altered concentrations often have been found in several neurological disorders and it has been suggested that, e.g., decreased levels of calbindin and parvalbumin may lead to a failure of neuronal calcium homeostasis and increased vulnerability to excitotoxicity.

Proposed Course of the Project: To continue studies of calretinin colocalization in various parts of the brain and periphery.

Publications:

Gulyas, A.I., Miettinen, R., Jacobowitz, D.M. and Freund, T.F.: Calretinin is present in non-pyramidal cells of the rat hippocampus: I. A new type of neuron specifically associated with the mossy fibre system. Neuroscience 48:1-27, 1992.

Miettinen, R., Gulyas, A.I., Baimbridge, K.G., Jacobowitz, D.M. and Freund, T.F.: Calretinin is present in non-pyramidal cells of the rat hippocampus: II. Coexistence with other calcium binding proteins and GABA. Neuroscience 48:29-43, 1992.

Ichikawa, H., Jacobowitz, D.M., Winsky, L., and Helke, C. J.: Calretinin-immunoreactivity in vagal and glossopharyngeal sensory neurons of the rat: Distribution and coexistence with putative transmitter agents. Brain Res. 557:316-321, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00396- 14 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular Biological Studies of Calretinin		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> <div style="width: 40%;"> Dr. Kenneth I. Strauss Dr. David M. Jacobowitz </div> <div style="width: 40%;"> IRTA Fellow Chief, Section on Histopharmacology </div> <div style="width: 20%; text-align: right;"> LCS NIMH LCS NIMH </div> </div>		
COOPERATING UNITS (if any) 		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Histopharmacology		
INSTITUTE AND LOCATION National Institute of Mental Health, Room 3D-48, Building 10, Bethesda, MD		
TOTAL MAN-YEARS: <div style="text-align: center;">1.6</div>	PROFESSIONAL: <div style="text-align: center;">1.3</div>	OTHER: <div style="text-align: center;">.3</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <div style="text-align: center;"> <p>In order to explore the role of <u>calretinin</u> in the brain and its regulation at the molecular level, we have cloned and analyzed the rat <u>cDNA</u> coding for calretinin. An immunoreactive clone was isolated from a rat brain cDNA expression library in λgt 11. The 1.45 kb insert was subcloned into the Eco RI site of the pGEM-4Z transcription vector for further analysis. Its identity was confirmed by comparison with human calretinin. The rat cDNA sequence comprised a 54 bp 5' untranslated region, an 816 bp open reading frame (ORF;) including start and stop codons, and a 579 bp 3' untranslated region. The ORF has 271 codons coding for a putative protein of 31.4 kDa. A polyadenylation signal and 13 adenylate residues were found near the 3' end.</p> <p>The evolutionarily conserved calcium binding domains and connecting regions and the limited changes observed between rat and chick primary structure lead us to believe that calretinin interacts with other highly conserved constituents of brain cells. This claretinin cDNA clone provides a new probe for the analysis of specific neurons in the central nervous system. The cDNA probe will allow a more detailed analysis of calretinin expression in the brain and will be useful for screening genomic libraries for the complete chromosomal gene.</p> </div>		

PROJECT DESCRIPTION:

Objectives: (1) To screen a λ gt 11 rat brain expression library with a calretinin antibody in order to isolate and sequence a rat cDNA clone coding for calretinin.

Methods Employed: (1) Immunological screening of a rat brain expression library. (2) Clone analysis, subcloning and sequencing by the dideoxynucleotide chain termination method.

Major Findings: (1) We have cloned and analyzed a rat cDNA coding for calretinin; (2) The 1.45 kb insert was subcloned into the Eco RI site of the pGEM-4Z transcription vector for further analysis. Its identity was confirmed by comparison with human calretinin. The rat cDNA sequence was comprised of a 54 bp 5' untranslated region, an 816 bp open reading frame (ORF) with start and stop codons, and a 579 bp 3' untranslated region. The ORF has 271 codons coding for a putative protein of 31.4 kDa. A polyadenylation signal and 13 adenylate residues were found near the 3' end; (3) The nucleotide and amino acid sequences in the proposed coding region were remarkably homologous to human calretinin (94.3% and 99.6% respectively), and chick calretinin (79% and 87% respectively). Rat and human untranslated regions were well conserved, while little homology was found between rat and chick (Table 1). Homology in untranslated sequences indicates selective advantage and therefore functional significance; (4) Only 4 amino acid differences, situated between the fifth and sixth calcium binding domains, were found between the inferred human and rat coding regions. Two of the changes were identical to chick calretinin, while the other two matched analogous amino acids in rat and human calbindin. There were 36 acid amino differences between rat and chick calretinin, 5 matched the analogous amino acids of rat calbindin. Comparison between rat and chick codons revealed that a central region (amino acids 67 to 204) comprising 51% of the protein contained only 19% of the amino acid changes. Conservative and nonconservative amino acid differences were randomly distributed between calcium binding and connecting domains.

Significance to Biomedical Research and the Program of the Institute:

Calcium binding proteins such as calretinin, calbindin, and calmodulin are thought to regulate many cellular activities in the CNS, such as electrophysiological actions, release of neurotransmitters, axonal flow, calcium transport and buffering. Recently, calcium binding proteins have attracted interest, since altered concentrations often have been found in several neurological disorders, and it has been suggested that decreased levels of calbindin D-28k may lead to a failure of neuronal calcium homeostasis and increased vulnerability to excitotoxicity. Molecular biological studies will allow a more detailed analysis of calretinin expression in the brain.

Proposed Course of the Project: To screen a rat cosmid library in order to obtain the genomic calretinin sequences. Such clones will reveal promoter and possibly enhancer regulatory elements. Expression of reporter gene constructs using the calretinin promoter regions, in the context of pharmacological responses *in vivo* or *in vitro*, will allow us to explore the functionalities of this neuron-specific protein with respect to those conserved components with which it interacts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02565-02 LCS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Calretinin-containing Neurons and Excitotoxic Injury		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="text-align: center;">Dr. David M. Jacobowitz Chief, Section on Histopharmacology LCS, NIMH</div>		
COOPERATING UNITS (if any) Dr. Gail Zeevalk Rutgers University of Medicine and Dentistry of New Jersey		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Histopharmacology		
INSTITUTE AND LOCATION National Institute of Mental Health, Building 10, Room 3D-48, Bethesda, MD.		
TOTAL MAN-YEARS: .5	PROFESSIONAL: .2	OTHER: .3
CHECK APPROPRIATE BOXES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>Uncontrolled Ca²⁺ flux is thought to orchestrate cell death due to <u>excitatory amino acids</u>. Studies suggest a relationship between <u>calcium-binding proteins</u> (CaBPs) and resistance to excitotoxicity. It is not clear, however, if neurons survive due to an absolute resistance to the excitotoxin. For example, in <u>chick retina</u>, <u>NMDA</u> kills most amacrine and some ganglion cells, whereas other retinal populations are unaffected. The resistance of neurons in the outer layers of retina is due to the absence of NMDA receptors on these cells. <u>Amacrine neurons</u> in retina are ideal to study the relationship between CaBPs and excitotoxicity because most amacrines are sensitive to NMDA in a dose dependent manner and the CaBPs, <u>parvalbumin</u> (PV); <u>calretinin</u>, (CR); and <u>calbindin</u> (CB) are found in this layer. Exposure of embryonic day 19 chick retina for 60 min to either 25, 100, 250 or 500 μM NMDA caused a dose dependent increase in LDH release measured after 24 hr of recovery. At 24 hr, retina was fixed and processed for PV, CR and CB immunoreactivity. The number of amacrine cells positive for the CaBPs were counted and correlated to <u>LDH</u> release. Statistical analysis showed a negative correlation between NMDA mediated LDH release and loss of PV + amacrines and no correlation with loss of CR or CB + amacrines. Thus, as LDH increased, the number of PV cells, but not CB or CR cells declined. Exposure to 500 μM NMDA for 24 hr resulted in a near total loss of PV and CB and 66% of CR + amacrines. These data suggest that CB and CR positive amacrine cells show a relative resistance to NMDA.</p>		

PROJECT DESCRIPTION:

Objectives: To study the influence of NMDA on calretinin-containing cells in the embryonic chick retina.

Methods Employed: (1) Incubation of embryonic chick retinas in physiological solutions, (2) Immunocytochemistry using the peroxidase procedure.

Major Findings:

- (A) Exposure of embryonic day 19 chick retina for 60 min to either 25, 100, 250 or 500 μ M NMDA caused a dose dependent increase in LDH release measured after 24 hr of recovery.
- (B) At 24 hr, retina was fixed and processed for parvalbumin (PV), calretinin (CR), and calbindin (CB) immunoreactivity. The number of amacrine cells positive for the calcium binding proteins were counted and correlated to LDH release. As LDH increased, the number of PV cells, but not calbindin or calretinin cells declined.
- (C) Exposure to 500 μ M NMDA for 24 hr resulted in a near total loss of PV and CB and 66% of CR positive amacrine cells.

Significance to Biomedical Research and the Program of the Institute:

Considerable evidence that excitatory amino acid systems are related to a number of major neurodegenerative diseases has added great impetus to research in this field. The present work demonstrates that calretinin neurons are relatively resistant to neurotoxic doses of excitatory amino acids compared to calbindin and especially parvalbumin. This supports the notion that calretinin also serves to buffer the inflow of calcium caused by a variety of toxins and thereby protects the cell from neuronal degeneration.

Proposed Course of the Project: To continue *in vitro* preparations and cells in culture (cerebellar granule cells) with excitatory amino acids.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00153 15 CHP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Treatment of Obsessional Children and Adolescents with Clomipramine

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M.D., Chief, CHP/DIRP/NIMH (others listed on next page)
Susan E. Swedo, M.D., Sr. Staff Fellow
Henrietta L. Leonard, M.D., Sr. Staff Fellow
A.J. Allen, M.D., Sr. Staff Fellow
Xavier Castellanos, M.D., Sr. Staff Fellow
Charles T. Gordon, M.D., Sr. Staff Fellow

COOPERATING UNITS (if any)

NIMH; NIA; NINCDS; NIH CC; Brown University; University of Michigan; Rockefeller Institute; Western Carolina Center

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bldg. 10, Room 6N240, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

3.25

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A two- to seven-year prospective follow-up study of 54 children and adolescents with Obsessive Compulsive Disorder (OCD) found 43% still met diagnostic criteria for OCD. Males with early onset of OCD were highly likely to have Tourette's Disorder. Short-term control of symptoms with clomipramine predicted better long-term outcome as did psychiatrically normal parents and family involvement in community activities.

A prospective follow-up of 16 women with trichotillomania found similar improvement from baseline as a group but about half of the subjects still having symptoms.

A new sample of 12 children with Sydenham's chorea finds that obsessive compulsive symptoms are present in 80%, and the severity of these symptoms parallel motor abnormalities.

Associate Investigators:

NIMH/DIRP/CHP: Markus J.P. Kruesi, Staff Psychiatrist, Kathleen M. McKenna, Sr. Staff Fellow; NIMH, CN: Dennis Murphy, M.D.; NIMH, NSB: David Pickar, M.D.; NIA, LN: Mark Shapiro, M.D., Pietro Pietrini, M.D.; NINDS: Jordon Grafman, Ph.D., CN, Collette Parker, M.D., Rafi Schifman, M.D.; NIDCD, IR: Christy Ludlow, Ph.D.; NIH, CC, DTM: Susan Leitman, M.D.; Brown University: Louise Kiessling, M.D.; University of Michigan: John Fink, M.D.; Rockefeller Institute: John Zabrowski, M.D., Ph.D.; Western Carolina Center: Tim Crawford, Ph.D., Jim Bodfish, Ph.D., James Madison, M.D.

OBJECTIVES:

To examine the natural history, biological correlates, associated disorders, and family psychopathology of obsessive compulsive disorder (OCD) and treatment response of obsessive compulsive (OC) "spectrum" disorders in children and adolescents.

MAJOR FINDINGS:

Follow-up Study

Fifty-four consecutive pediatric patients with OCD who participated in controlled clomipramine (CMI) trials and then received a variety of interim treatments, were reevaluated two- to seven-years after initial contact. On follow-up, 23 (43%) of the subjects still met diagnostic criteria for OCD, and only 6 (11%) were totally asymptomatic, supporting previous reports of the chronicity and intractability of the illness. Thirty-eight subjects (70%) were on psychoactive medication at the time of follow-up; including 3 of the 6 asymptomatic patients. Although OC symptomatology continued, the group was improved at follow-up, with only 10 (19%) subjects unchanged or worse. A more severe OCD symptomatology score after 5 weeks of CMI, lifetime history of a tic disorder, and presence of parental Axis I psychiatric diagnosis predicted higher OCD symptom score at follow-up. Thus with intensive treatment, most pediatric patients can expect substantial improvement but not complete remission with time.

Pharmacotherapy

In collaboration with Dr. David Pickar, Clinical Neuroscience Branch, NIMH, we are enrolling subjects in a challenged study using D-cycloserine. We hypothesize that there will be an immediate, but short-lived improvement of OCD symptomatology, mediated through the dopaminergic system. Of the 4 subjects completed to date, one subject experienced a dramatic improvement on 15mg of D-cycloserine. When the trial was repeated, he responded to 250mg of D-cycloserine. Extended blinded trials of 3 days of 15mg, 250mg, and placebo yielded a positive clinical response to 15mg D-cycloserine.

Open trials of fluvoxamine in CMI and fluoxetine non-responders (or those who could not tolerate the medication) with the participation of 8 adolescents and adults. Of the 4 completed this year, only one individual had a positive therapeutic response.

Family Environment of OCD

Several measures of family environment, functioning and psychopathology were administered at baseline to the parents of 47 children and adolescents with OCD. These family measures included structured interviews for DSM-III Axis I disorders, the Family Environment Scale (FES), the Dyadic Adjustment Scale (DAS) of marital adjustment, Expressed Emotionality (EE), and the Buss-Durkee Hostility Inventory (BDI). All patients participated in controlled studies of CMI and were subsequently reevaluated 2 to 7 years later. Several subscales of the FES and BDI, including measures of family independence, social activity, and parental hostility, were found to predict short- and long-term outcome, and future psychiatric hospitalization. The DAS, EE, and family psychopathology were not found to predict outcome when analyzed along with the FES and BDI in multivariate analyses, nor did these results appear to be due to confounding demographics, differences in baseline severity, or treatment compliance. A multidimensional biopsychosocial model of OCD is supported.

Trichotillomania

To explore a possible relationship between trichotillomania, (TTM) (compulsive hair pulling) and OCD, 65 out of 69 (94%) first-degree relatives of 16 female probands with severe chronic TTM were compared with two control groups for OCD and for TTM. Three (19%) of the 16 TTM probands had at least one first-degree relative with a lifetime history of OCD, and there was an age corrected rate of 6.4% of first-degree relatives with OCD. No relative in control group A met criteria for OCD. There was a trend (Fishers exact $p = .07$, two tailed) for a higher rate (age corrected) of OCD in TTM families; these pilot data are consistent with the concept of a spectrum of obsessive compulsive disorders which includes TTM and other pathological grooming behaviors.

Phenomenologic and pharmacologic investigations of males with TTM demonstrated similar psychopathology and treatment response as for previously studied female subjects.

Four- to five-year follow-up of the first 16 women with TTM revealed that, as a group, they remained improved from baseline. The mean percent improvement was 40% with half of the subjects reporting moderate to complete symptom remission. No baseline predictors of continued hair-pulling were identified.

Early-onset TTM patients are being recruited and followed in a naturalistic study. Preliminary results with 12 patients suggest an episodic course is present; in two cases, we have demonstrated that these recurrences coincided with increased titers of antibodies directed against streptococcal bacteria.

Sydenham's Chorea

Prospective phenomenologic examination of 12 children with Sydenham's chorea revealed presence of new obsessive compulsive symptoms in 80% which peaked in severity as movements worsened and remitted as chorea resolved. In addition, previous reports of emotional lability were confirmed. Antineuronal antibody measurements were overwhelmingly positive in these patients and preliminary results demonstrate that the antibodies are absorbed by lyophilized streptococcal bacteria.

A controlled treatment comparison of intravenous immunoglobulin, plasma exchange, and prednisone for Sydenham's chorea is underway. Standardized videotaped exams will be "blindly" rated to assess improvement and rapidity of remission. It is anticipated that both plasma exchange and IV IG will be superior to prednisone and placebo. In addition, plasmapheresis will be performed at baseline on all patients to obtain sufficient quantities of plasma for documentation of specific antibodies.

Stuttering

Thirteen adults with developmental stuttering (moderate- to severe-degree) have completed a 10-week double-blind CMI/desipramine (DMI) comparison following a 2-week single-blind placebo washout.

Self report ratings of stuttering severity and avoidance in the first ten subjects show improvements on clomipramine as compared to desipramine with a trend towards statistical significance ($p = .07$).

Speech pathologists blindly rate percent dysfluency, number of dysfluent events, and speech rate during four speaking conditions: reading or repeating sentences (depending on which is more dysfluent); communicating how to construct a block design; making a telephone call; and speaking in front of a small audience (5-8) people. Preliminary analysis of the audience condition in the first six subjects reveals no specific pattern of response to clomipramine and desipramine.

There have been no serious adverse effects with either CMI or DMI.

Over the next six months, two more subjects will complete the CMI/DMI comparison and the self-report and objective speech ratings will be analyzed. Drug response will be correlated with stuttering severity, degree of obsessive-compulsive symptomatology (measured by a Leyton obsessive inventory), plasma drug level, and MRI variables.

METHODS EMPLOYED:

Phenomenological studies of children and adolescents with Segawa's Dystonia and Sydenham's chorea are ongoing to explore the association of these presumed basal ganglia disorders of childhood and OCD.

Double-blind comparison studies of DMI and CMI have been conducted with putative "OC spectrum" disorders including: stuttering, autistic behaviors, compulsive shopping, paraphiliac, and nail biting. A study of sertraline in dogs with acral lick is planned.

Follow-up studies of a community sample of untreated OCD and a treated clinical sample are underway.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

OCD is thought to affect four million persons in this country alone. Most have never been treated and are unaware that they have a diagnosable, let alone treatable, disorder. Until recently, there has been little research on this disabling disorder.

PROPOSED COURSE OF PROJECT:

A major effort studying Sydenham's Chorea as a model of OCD will occupy much of the next two years. Subjects are randomized to either prednisone or intravenous immunoglobulin plasma exchange treatments. Antistreptococcal antibodies will be followed in relation to neurological and psychiatric status.

Other childhood onset anxiety disorders will be studied in a trial of serotonin uptake inhibitors for Elective Mutism and Social Phobia.

The study of repetitive unwanted behaviors in the mentally retarded is proposed as an Off-Site Project to be conducted at the Western Carolina Center, Morganton, NC.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH-00178-11 CHP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Brain Structure & Function in Developmental Neuropsychological Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith M. Rumsey, Ph.D., Judith L. Rapoport, M.D. (others listed on next page)

COOPERATING UNITS (if any)

Clinical Brain Disorders Branch/NIMH; Sect on Clin. Brain Imaging, LCM/NIMH; LPP, NIMH; CADRD/NIMH; Clinical Brain Research, NIAAA;

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bldg. 10, Room 6N240, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

6.0

PROFESSIONAL:

3.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

MRI studies of neuroanatomical correlates of developmental disorders and normal development are currently underway. To date, MRI findings suggest decreased frontal and temporal lobe volumes in severe conduct disorder patients and increased caudate size in patients with Sydenhams chorea. Ongoing MRI studies show trends for reduced midsagittal area measurements of the corpus callosum in ADHD patients and a reduction in the midsagittal area of the brainstem and pons in schizophrenic patients.

O-15 PET and MRI studies of OCD are currently underway to test hypotheses of whether generalized anxiety, disgust and obsessional anxiety are different as evidenced by differences in regional cerebral blood flow and if imaginal exposure and in vivo exposure to obsessions are different in the physiological response elicited. Specific ROIs of interest include the anterior cingulate, orbital frontal cortex, and caudate.

O15 PET studies of seven calendar calculating savants and 14 normal controls show higher left parietal blood flow at rest than controls. Analysis of activation date to date indicate that during calendar calculation, savants have less left frontal and more right parietal blood flow change.

Associate Investigators:

NIMH, DIRP, CHP: B.J. Casey, Ph.D, Staff Fellow, Jay Giedd, M.D., Sr. Staff Fellow, Kathleen McKenna, M.D., Sr. Staff Fellow, Charles T. Gordon, M.D., Sr. Staff Fellow; NIMH, LCM: Robert Cohen, M.D., Alan Zametkin, M.D., NHLBI, NIH: Robert Turner, Ph.D., University of Georgia: Manny Casanova, M.D., Ph.D.; Carnegie Mellon: Jonathan Cohen, M.D., Ph.D.

OBJECTIVES:

To identify functional and anatomical correlates of developmental disorders, to identify functional and anatomical correlates of exceptional, deficient, and normal development, and to further develop neuroimaging techniques for utilization with these developmental populations (e.g. functional MR).

MAJOR FINDINGS:

PET Savants

PET scans have been collected on 7 calendar-calculating savants and 14 normal controls. MRI scans have been obtained on these same subjects as well as an additional 4 savants.

The PET data is in the final stages of analysis, having been analyzed with our old regions of interest approach as well as with Statistical Parametric Mapping (SPM). A systematic comparison of these two data analytic approaches is underway.

SPM offers advantages of stereotaxic normalization. Each scan is rotated, resized and reshaped to a template PET scan with the orientation and dimensions of a Talairach stereotaxic brain atlas. Between-scan and between-subject differences in global blood flow are removed using analysis of covariance. Comparisons (between group and group x state) are then computed using a t statistic on a pixel-by-pixel basis. Significant pixels (reflecting significant within-subject activations or group differences) are mapped onto brain images. Coordinates for the significant regions of difference referenced to the Talairach atlas are given. Overall significances are examined by comparing the numbers of significant pixels to the number expected by chance using a chi square statistic.

While the mechanics of the SPM analysis are complete, interpretation has just begun. Therefore, only the results of the region of interest analysis are included below.

At rest, the savants show higher left parietal blood flow and lower right and left hippocampal flow relative to controls.

Perhaps the most consistent difference in activation thus far appears to be that of less left frontal activation in savants. This difference was seen on both the calendar calculating and the mental addition tasks, during which savants showed no significant regions of activation.

In going from rest to calendar calculating, controls activated superior right parietal and left frontal cortex, while savants showed no significant regions of activation. Savants showed significantly less activation than controls in left frontal cortex, but more activation than controls in one right parietal (though this region was not significantly activated in savants considered separately). These results represent a diminution of our earlier findings with n's of 3 and 6 and may reflect improvement in the control's group calendar calculating skill, an interpretation subject to further testing using correlations between performance and blood flow.

On mental addition, the controls activated left and right frontal, right parietal and left temporal cortex, while savants again showed no regions of significant activation. Group differences in activation reached significance in left frontal and temporal and in right temporoparietal cortex, where controls showed greater activation than savants. Again, this might reflect increased effort and less automaticity on the part of savants, an interpretation subject to further examination using correlations with performance and other behavioral variables.

Despite less than normal hearing in three savants, these sensory deficits do not appear to have affected our PET data. Few group differences were seen in primary auditory cortex during "speech noise", a fluctuating signal of speech frequencies. Likewise, group differences seen when comparing the rest with speech noise do not involve primary auditory cortex. This gives us confidence that differences in activation on our tasks cannot be attributed to primary auditory deficits in the savants.

Neuropsychology Savants

Neuropsychological examination of 11 savants with pervasive developmental disorders (8 autistic, 3 pervasive developmental disorder-not otherwise specified) indicated Full Scale IQs ranging from 65 to 107, lending some confirmation to the fact that such skills are seen primarily in high-functioning patients. Thus the term "idiot savant" is a misnomer.

Uneven cognitive profiles, similar to those reported in high-functioning autistic patients without savant skills, were noted. Deficits in executive functions dependent on the integrity of the frontal lobes and strengths involving spelling, word recognition, digit span, spatial skills, and musical aptitude, skills subserved by posterior association cortex, were seen.

To address the issue of whether such skills represent neuropsychological "superiorities" (skills superior to those of the general population of merely "relative strengths" (strengths relative to the patient's deficits), performance in various skill domains was compared with published norms and with each patient's IQ. Superior skills, seen in four, and relative strengths, seen in seven, were heterogeneous, but most consistently involved skills thought to be subserved by right posterior cortex, such as pitch discrimination and tonal memory.

To discern patients' reliance on memory based approaches and rule-based strategies, a test of calendar calculating skill was devised in which subjects were given dates and asked to provide the corresponding day of the week on which they fell. When accuracy and reaction times were compared for past versus future dates and dates in certain years that represented exact replicas of other years, results supported reliance on a memory-based approach. Correlations of calendar test performance with neuropsychological tests suggest facilitation by good immediate auditory memory, good freedom from distractibility and knowledge of mathematical rules.

Attentional Dysfunction Savants

A series of computerized tasks were designed to test a hypothesis of dysfunctional attention systems in the brain resulting in deficient orienting and overfocused attention. There were four general findings. First, the savants and controls did not differ in their ability to detect a rare visual target. Second, the savants were unable to efficiently divide their attention between two simultaneous detection tasks that required flexible shifting from one modality to another. Third, the savants detected fewer auditory targets than the controls. Finally, deficient orienting or a deficit in the ability to shift attention was observed in overall

slower reaction times on tasks that required redirecting of attention from one spatial location to another, especially, when shifting to the left visual field implicating right parietal dysfunction.

MRI Conduct Disorder

Preliminary results from MRI data analyses of 10 severe conduct disorder patients and 10 normal controls indicated that the conduct disorder patients had a significant decrease in frontal lobe volume and right temporal lobe volume. These results are consistent with reports of sociopathic behavior following frontal lobe lesions, and association with EEGs in the temporal lobe and limbic regions.

MRI Sydenham's Chorea

MRI scans of 4 male and 6 female patients and 10 sex and age matched controls showed an increase in the size of the caudate nucleus, hypothesized to be from edema secondary to post streptococcal autoantibodies directed against receptors in the caudate.

MRI Dyslexia

MRI measures completed to date on 15 severely dyslexic men and controls include temporal lobe volumes, height of the Sylvian point, depth of the supratemporal plane both anterior and posterior to the internal auditory canals and anterior, middle and posterior corpus callosum. Though data analysis is still in progress, an interim analysis suggested an increase in the size of the posterior corpus callosum as predicted. Such an increase suggests increased axonal connections between homologous posterior brain regions. This might reflect an increase in the size of the right planum temporale, reported previously in postmortem and MRI studies of dyslexia.

PET Dyslexia

PET data on 15 severely dyslexic men and 25 controls indicate a failure of the dyslexics to activate left temporoparietal cortex during a phonologic, rhyme detection task, normal activation of frontotemporal language cortex in the left hemisphere during sentence comprehension, and decreased right frontotemporal activation during tonal memory. This study is the first to demonstrate the hypothesized dysfunction of left temporoparietal regions near the angular/supramarginal gyri. This is likely due to our use of appropriate activation paradigms in conjunction with PET. These findings suggested more focal left sided dysfunction in dyslexia than previously hypothesized.

Of particular interest is the finding that the degree of right temporal activation is significantly and positively correlated with all six of our "dyslexia-related trait" variables (which include a variety of reading and spelling measures). Also of interest is the highly significant performance deficit seen in our dyslexics on our tonal memory task ($p < .01$), a finding that parallels Dr. Tallal's findings in dyslexic children. These findings suggest that difficulties in rapid temporal processing in dyslexia are not restricted to linguistic stimuli, but rather are more generic, and that these deficits are attributable to right-sided dysfunction. Several investigators have suggested that such deficits are attributable to the left hemisphere's predilection for processing rapid sequential stimuli, but our data contradict this.

Should our MRI findings include right-sided abnormalities, suggesting an increase in the size of the right planum or temporal lobe, as reported in autopsy studies, we will examine anatomical and functional relationships statistically.

MRI Normal Volunteers

The relation between age, gender, and pubertal status on hippocampal, frontal cortex, and basal ganglia anatomy, and myelination in normals is underway. To date, 22 males and 12 females of the target number of 45 males and 45 females between the ages of 5 and 18 years have been scanned.

Preliminary MRI results on 14 normal children between the ages of 8 and 16 years indicate that performance on selective attention tasks, but not divided attention tasks are correlated with the size of the anterior cingulate, especially the right anterior cingulate even when brain size, IQ, and age are covaried. This structure/function relationship is consistent with the most recent findings in the adult literature using PET and will be examined further in clinical disorders with attention deficits (e.g., schizophrenic and ADHD patients).

MRI ADHD

Preliminary MRI analysis are underway to examine frontal lobe myelination, frontal lobe volume, corpus callosum size and shape, and basal ganglia structures, all of which have been implicated in ADHD. Preliminary results from 10 ADHD males and 9 age-age sex-matched controls indicate a trend toward a reduced area measurements for the corpus callosum, especially in the anterior (genu) and posterior regions (splenium).

MRI analyses of ADHD patients will be compared with two other related disorders: ADHD patients with Tourette's Syndrome and Gifted ADHD patients. The target number of each group of males with age-and sex-matched normal controls. To date, 4 male patients with ADHD and Tourettes have been scanned and 1 male gifted ADHD patient.

MRI Childhood Onset Schizophrenia

MRI analysis of 20 patients and 20 controls between the ages of 5 and 18 will examine regions of the brain implicated in schizophrenia, including the hippocampus, frontal lobes, corpus callosum, and brainstem areas. Preliminary MRI analysis of 3 male and 2 female patients and 3 sex-and age-matched controls indicate a reduction in the area of the pons and a trend towards a reduction in the midsagittal area of the brainstem.

PET OCD

Ongoing O-15 PET activation studies of 15 obsessive compulsive patients are testing hypotheses of whether generalized anxiety and obsessional anxiety are different as evidenced by differences in regional cerebral blood flow and if imaginal exposure and in vivo exposure to obsessions are different in the physiological response elicited. Specific ROIs of interest include the anterior cingulate, orbital frontal cortex, and caudate, all of which have shown unusual hypermetabolic activity in PET studies with OCD patients.

O-15 PET activation studies of 15 OCD patients and 15 controls are testing hypothesis of whether normals and patients differ in regional cerebral blood flow patterns in response to anticipatory anxiety, "universal" disgust, and during neuropsychological tasks on which OCD patients show deficits.

MRI OCD

MRI data analyses for the total sum of 30 OCD patients and 15 normals will examine differences in size and shape of the anterior cingulate, orbital frontal cortex, and caudate between the two groups. Additionally, correlative analysis will examine the relation between these anatomical measures and neuropsychological measures.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH

Childhood onset psychiatric illnesses represent a significant proportion of mental illness in the United States. This study continues to identify and localize pathological processes that we hope will lead to further understanding of the etiology and advancement in the treatment of childhood onset psychiatric illness.

The identification of neuroanatomical correlates of neuropathology in a variety of childhood onset psychiatric illnesses including attention deficit-hyperactive disorder, obsessive-compulsive disorder, childhood onset schizophrenia, dyslexia, Sydenham's chorea and autism is well in the works. Functional neuroimaging with PET has provided the best means for localizing cerebral dysfunction, while quantitative MRI has provided information on neuroanatomical abnormalities in the size and shape of brain structures. Together, these neuroimaging techniques may reveal compensatory or residual function of affected structures during development.

PROPOSED COURSE OF PROJECT

Although PET currently provides the best means for localizing cerebral dysfunction, our studies have been limited to adolescent and adult patients with childhood onset psychiatric illnesses. Radiation exposure inherent in this traditional neuroimaging technique of which developmental populations are most vulnerable. Therefore, neuroanatomical development, both pathological and normal, has been an understudied area. With recent developments in magnetic resonance, a completely non-invasive imaging technique, we will have the capability of dynamic MR imaging in developmental patient populations and normals. This will compliment our growing "library" of MRIs on a growing number of developmental disorders and will enable localization of pathological processes as well as anatomical abnormalities.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH-02240-08 CHP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Disruptive Behavior Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M.D., Chief, CHP/DIRP/NIMH (others listed on next page)

COOPERATING UNITS (if any)

NIMH, LCS; NIMH, LPP; Nathan Kline Institute; Tufts University

LAB/BRANCH

Child Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Building 10, Room 6N240, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A two-year prospective follow-up of 29 (100%) children and adolescents with disruptive behavior disorders found that baseline lumbar CSF monoamine metabolite concentration and autonomic nervous system reactivity had significant predictive value (Kruesi et al, 1992). 5-HIAA concentration significantly predicted severity of physical aggression during follow-up. Skin conductance level significantly predicted institutionalization. CSF HVA predicted suicide attempts. The direction was as predicted, with lower CSF 5-HIAA and lower autonomic activity correlated with poor outcome. Moreover, when hierarchically entered into multiple regression and discriminant function analyses after a variety of other non-laboratory predictors, CSF and autonomic measures still contributed significantly to the variance.

Preliminary studies of stimulant drug response of patients with Hyperactivity and Tourette's disorder indicates that not only do some patients' tics not worsen on stimulants, but that over time and/or increase in dose, tics may even improve.

Associate Investigators:

NIMH, DIRP, CHP: F. Xavier Castellanos, M.D., Sr. Staff Fellow, Jay Giedd, M.D., Sr. Staff Fellow, B.J. Casey, Ph.D., Staff Fellow, Markus J.P. Kruesi, M.D., Staff Psychiatrist; **NIMH, LCS:** William Z. Potter, M.D., Ph.D.; **NIMH, LP:** Theodore Zahn, Ph.D.; **Nathan Kline Institute:** Thomas Cooper, M.A.; **Tufts University:** David Feldman, Ph.D.

OBJECTIVES:

1. To study the pathophysiology of disruptive behavior disorders.
2. To determine whether biologic measures are useful in predicting outcome and/or classification of children and adolescents and/or stimulant drug response in this heterogeneous group.
3. To examine the response to stimulants of Tics, OCD and ADHD in appropriate pediatric populations.
4. To examine ADHD in gifted and talented populations.

MAJOR FINDINGS:

A 2.3 year prospective follow-up of 29 (100%) children and adolescents with disruptive behavior disorders found that both baseline lumbar CSF 5-HIAA and HVA concentrations as well as autonomic measures contributed significantly to the prediction of outcome in expected directions with lower concentrations associated with more aggression directed at others, suicide attempts.

Magnetic resonance imaging techniques have been used to investigate neuroanatomic differences between children and adolescents with or at high risk for antisocial personality disorder. Findings from a group of 10 patients and 10 age, sex, handedness and race matched controls are in the final stages of analysis (Kruesi et al, manuscript in preparation). The findings have particular importance because of criticism of the Antisocial personality disorder diagnosis as an attempt to "medicalize" bad behavior. The localization of neuropathology promises to have significant impact, in furthering research and in urging compassion for children and adolescents with a costly and unpleasant disorder.

Comparison of CMI and DMI in paraphilics showed that there was not selective advantages to the more potent serotonin reuptake inhibitor (Kruesi et al, in press). This suggests the paraphilias which are often termed compulsive sexual behaviors are unlike obsessive compulsive disorder. The demonstration of benefit from antidepressants over placebo in paraphilia is a first and suggests further research.

To date, eight boys with ADHD and Tourette's Disorder have completed a double-blind trial of methylphenidate, dextroamphetamine and placebo. Preliminary analysis of the first six patients showed that while motor and vocal tics increased initially on both stimulants, they decreased towards placebo level for 6 out of 6 children on methylphenidate, and for 3 out of 6 on dextroamphetamine at the highest dose tested. Long-term follow-up shows that 5/6 continue to derive significant benefit from stimulant treatment, with acceptable effects on tics.

Intellectually gifted children who also have ADHD have not been previously studied in a systematic manner. Our analysis of 72 ADHD children showed that 13 had WISC-R IQ > 119. These children had a greater improvement in hyperactivity on dextroamphetamine than did the average IQ subjects. This did not hold for methylphenidate. Eleven of the thirteen were discharged on dextroamphetamine, with drug chosen while still blind.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Disruptive behavior disorders are the most frequent presentation of children and adolescents for psychiatric services. The findings of prospective prediction from the CSF markers adds consistency to the findings in adult patient groups. This adds additional impetus for basic scientists to discover the biological basis of spinal fluid monoamine metabolite concentrations.

At least 50% of boys with Tourette's Disorder also have ADHD. Frequently it is the symptoms of inattention, impulsivity and hyperactivity that are the most troublesome. Due to the erroneous conclusion that stimulants produce Tourette's Disorder *de novo*, most clinicians do not use them in this population, although they are the most effective drug therapy. The work done here helps to open up the range of therapeutic options for children and adolescents with otherwise intractable behavioral disorders.

Intellectually gifted children with ADHD are very often undiagnosed. They are usually able to meet minimum school requirements, and so are not classified as learning disabled by the school systems. Nevertheless, these children are at very high risk for underachievement, and for many of the sequelae associated with the disruptive behavior disorders. The findings of preferential response to Dexedrine in hyperactive children with IQ > 119, would if replicated, be clinically significant since this stimulant is rarely used.

PROPOSED COURSE OF STUDY:

Ongoing follow-up of the combined sample of 70 individuals for whom we have CSF monoamine metabolite concentrations and autonomic nervous system responsivity is planned and underway. Hyperactive Tourette's Disorder patients will continue to be studied to define patterns of stimulant drug response. Intellectually gifted hyperactive children are being recruited to confirm our preliminary findings of differential drug response and to further define the phenomenology of this unstudied subgroup. An additional 25 hyperactive children will be recruited to examine CSF predictors of response to pemoline.

PUBLICATIONS:

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Zametkin A, Andreason P, Kruesi M. Laboratory and diagnostic testing. In: Wiener JM, ed. *Textbook of child and adolescent psychiatry*. Washington, D.C.: American Psychiatric Press, 1991;121-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-MH-02581-02 CHP

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Childhood Onset Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Judith L. Rapoport, M.D., Chief, CHP/DIRP/NIMH (other personnel listed on next page)

COOPERATING UNITS (if any)

NSB, NIMH; CADRD, NIMH; CBD, NIMH; LPP, NIMH; Clinical Center Pharmacy, Pharmacokinetics Lab.; LCS, NIMH; LCM, NIMH; CNB, NINCDS; UCLA; Johns Hopkins University

LAB/BRANCH

Child Psychiatry Branch

SECTION

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TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

2.25

OTHER:

2.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Children and adolescents meeting DSM-III-R criteria for schizophrenia are available and eleven subjects have participated to date in this study of the phenomenology, neurobiology and pharmacologic response of childhood onset schizophrenia. Diagnostic reliability has been established. One hundred fifty children and adolescents with a clinical diagnosis of schizophrenia have been screened by medical records and 46 patients, appearing to meet DSM-III-R criteria for schizophrenia with medical record history of onset of psychosis prior to age twelve were interviewed personally. At least three subgroups of DSM-III-R diagnosed schizophrenic children have been hypothesized based on differences in clinical phenomenology.

Pilot family/genetic data indicate one case is familial and a second subject had a chromosomal aberration. Autonomic measures parallel adult schizophrenia MRI abnormalities have been found in the brainstem and [18] fluoro-2-deoxy-D-glucose (FDG) PET scanning indicates decreased metabolism in the left hippocampus with no hypofrontality. Safety and efficacy of clozapine in this age group is suggested by an open trial in eight subjects and a double-blind haloperidol/clozapine comparison in seven subjects.

In a double-blind comparison of desipramine and placebo, children with autistic (n=24) disorder show a selective response to clomipramine supporting a serotonergic disturbance in this disorder.

Associate Investigators:

NIMH, DIRP, CHP: Charles T. Gordon, M.D., Sr. Staff Fellow, Kathleen M. McKenna, M.D., Sr. Staff Fellow, F. Xavier Castellanos, M.D., Sr. Staff Fellow, Judith M. Rumsey, Ph.D., Guest Researcher; NIMH, NSB: David Pickar, M.D., Richard Owen, M.D., Robert Litman, M.D.; NIMH, LPP: Theodore Zahn, Ph.D.; NIMH, LCS: William Z. Potter, M.D.; NIMH, LCM: Alan J. Zametkin, M.D.; NIH, CC: Dale Grothe, Pharm. D., Janice Barnett, Ph.D.; NINCDS, CNB: Mark Hallett, M.D.; UCLA: Robert F. Asarnow, M.D.; Johns Hopkins: Ann Pulver, M.D.

OBJECTIVES:

To identify a group of children and adolescents with prepubertal onset of DSM-III-R schizophrenia, characterize them in terms of early development, clinical symptoms and comorbid diagnoses, illness course, genetics, neuropsychological profile, brain structure and function, neurochemistry and pharmacologic response to typical and atypical neuroleptics as well as investigate the relationship of childhood schizophrenia to autism spectrum disorders and later onset schizophrenia; also to identify serotonin relevant behaviors in autism and childhood onset schizophrenia.

MAJOR FINDINGS:

Although childhood onset schizophrenia is a relatively rare disorder, 12 subjects have been enrolled meeting DSM-III-R criteria for schizophrenia with onset of psychosis prior to the twelfth birthday. Reliability of diagnosis is being established.

Based on our clinical observations of the 46 children screened with a clinical diagnosis of schizophrenia, we hypothesize three subgroups of children meeting DSM-III-R criteria for schizophrenia. The first, relatively rare, group has features common with later onset schizophrenia and probably is on the same pathophysiologic continuum. A second, relatively common "organic" group is characterized by early onset (prior to age five years) of severe affective lability, aggressive outbursts, hyperactivity, inattention, multiple cognitive impairments, immature and overly-dependent social relationships, and suspiciousness with episodic hallucinations and seemingly "bizarre behavior". This second group of subjects lacks formal thought disorder, prominent or bizarre delusions, flat affect, social withdrawal and apathy commonly seen in the first group. A third group of autism-related "psychotic" children has abnormalities in reciprocal social interaction, affective response and repetitive behaviors of the type seen in pervasive developmental disorders, but magical thinking, bizarre behaviors (e.g. talking and laughing to self) and unusual perceptual experiences make differentiation from DSM-III-R schizophrenia difficult. Whether these subgroups are on an etiologic continuum or represent distinct categories of pathology will be addressed by the present study.

Pilot data is already available in several areas of study: Family/genetic studies show one of the 11 families to have multiple maternal relatives with psychotic illnesses and a second subject and several paternal relatives to have a translocation of chromosomes 1 and 7. Autonomic measures comparing the first five schizophrenics with thirty-four age and sex-matched normal controls, reveal increased baseline autonomic indices and decreased autonomic responsivity to innocuous and meaningful stimuli, similar to the pattern seen in acutely ill adult schizophrenics. Preliminary MRI findings (in five subjects and three age, sex,

and handedness matched normal controls) show smaller midbrain and pons areas on a midsagittal slice in the schizophrenics without significant shape differences. FDG PET studies in five subjects and five normal age- and sex-matched controls reveal decreased metabolism in the left hippocampus in the schizophrenics with no global or frontal differences.

Eight schizophrenic subjects, previously showing poor response to typical neuroleptics, have completed an open six-week clozapine trial and seven subjects have completed a double-blind haloperidol/clozapine comparison. Clozapine has been efficacious for both positive and negative symptoms and has been better than haloperidol with 7 of 9 subjects being much or very much improved on clozapine, as measured by the clinical Global Impressions Scale. Clozapine has been well tolerated with no serious adverse effects.

Children and adolescents with autistic disorder (N=24) have been shown to demonstrate selective response to clomipramine as compared to desipramine and placebo in a ten-week double-blind crossover study supporting a serotonergic disturbance in autism and are an important contrast group for the schizophrenics in terms of serotonin biochemistry, brain anatomy, language, and early development.

METHODS EMPLOYED:

The structured diagnostic interview used is a combination of the K-SADS and DICA. Early development is examined using the Autism Diagnostic Interview. Neurological evaluation includes examination for "soft signs" as well as motor trajectory and blink reflex testing. Family/genetic methods include pedigree construction, linkage studies of familial cases, karyotyping (including fragile X0 and establishment of permanent cell lines. Smooth pursuit eye movements are studied. Skin conductance and heart rate at baseline and in reaction to innocuous tones and in a reaction time task are the autonomic measures employed. The neuropsychological battery includes tests of IQ and academic achievement, language, right hemisphere function, attention and frontal lobe. Magnetic resonance imaging on a 1.5 Tesla scanner using a rapid sequence program under chloral hydrate sedation is used to study brain anatomy. Fluoro-2-dexoxy-D-glucose PET scanning is being performed in 12-18 year old schizophrenics and controls using a modified dosage regime to decrease radioactivity exposure. Cerebrospinal fluid is being obtained for neurochemical study by lumbar puncture during the medication-free phase and during treatment with both typical and atypical neuroleptics.

Rating instruments for the double-blind haloperidol/clozapine trial were chosen to reflect independent changes in positive and negative schizophrenic symptoms, autistic features, and nonspecific behavioral difficulties, as well as to assess the full range of side effects are: daily nurses' Bunny Hamburg Psychosis Ratings and Teacher's 10-item Conners; weekly nurses' Children's Psychiatric Rating Scale, Teacher's 39-item Conners, and physician ratings which include: Brief Psychiatric Rating Scale, Children's Brief Psychiatric Rating Scale, Schedule for the Assessment of Positive Symptoms, Schedule for the Assessment of Negative Symptoms, Bunny Hamburg Ratings of Psychosis, Depression, Mania and Anxiety, Children's Global Impression, Abnormal Involuntary Movements Scale, Simpson scale of Extrapyramidal Side Effects and the Subjective Treatment Emergent Symptoms Scale. Ratings in the clomipramine/desipramine crossover study for autistic disorder include: Children's Psychiatric Rating Scale-Autism Relevant Subscale, modified Comprehensive Psychopathological Rating

Scale-OCD Subscale and modified NIMH Obsessive Compulsive Ratings and Clinical Global Impression.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Schizophrenia affects approximately 1% of the population of the United States and is a major national health problem costing billions of dollars annually. Childhood onset schizophrenia, although relatively rare, results in lifelong disability and dysfunction for the patients and their families. In addition, understanding the basis for the early onset disorder will provide fundamental contributions to biological theories of the etiology of schizophrenia.

PROPOSED COURSE OF PROJECT:

We plan to increase the sample size in the childhood onset schizophrenia study to 30-35 subjects. After subjects complete the study, we will follow them at six month intervals for at least the next 10-15 years (into adulthood) with behavioral ratings as well as periodic MRI and PET scans to examine changes with brain maturation and development. We are expanding the clomipramine/desipramine trial in autistic disorder to include non-autistic mentally retarded children with stereotypies and other repetitive and "compulsive" behaviors to assess the specificity of clomipramine's efficacy to the autistic syndrome.

PUBLICATIONS:

Gordon CT: Childhood onset schizophrenia. In: Peschel E (ed) Schizophrenia in children and adolescents in biologically-based brain disease in children and adolescent. New Directions Series: Mental Health Services. San Francisco, Jossey-Bass, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00086-17 CNG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Outpatient Clinic for Genetic and Pharmacologic Studies of Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	E.S. Gershon	Branch Chief	CNG, NIMH
Others:	S. Simmons-Alling	Clinical Nurse Expert	CC, NIMH
	A.S. Robb	Medical Staff Fellow	CNG, NIMH
	A. Ram	Guest Researcher	CNG, NIMH
	M.E. Maxwell	Research Social Worker	CNG, NIMH
	J.J. Guroff	Research Social Worker	CNG, NIMH
	D. Kazuba	Research Social Worker	CNG, NIMH

COOPERATING UNITS (if any)

Department of Psychiatry, Jefferson University (W.H. Berrettini); Indiana University (J. Nurnberger); University of Utah (B. Byerley); Center for Behavioral Medicine (R. DuPont); University of Tel-Aviv (P. Sirotka)

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.5

PROFESSIONAL:

3.75

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Because the major psychiatric disorders are common disorders with complex inheritance, unless one collects large clinical samples, there are severe practical limits on investigators' ability to test hypotheses of genetic linkage or association. We have previously collected our own series of 20 bipolar manic-depressive (BP) families with 338 cell lines.

We are now engaged as the Intramural collaborators in an NIMH collaborative study, which will collect more than 150 families each with BP, schizophrenia (SZ), and Alzheimer's disease. Two interview instruments have been prepared for BP and SZ families: the Diagnostic Instrument for Genetic Studies (DIGS), and the Family Interview for Genetic Studies (FIGS). A reliability study has been submitted for publication. Collection of our Branch's pedigree series for this study has begun with 4 families collected.

We are also collecting bipolar pedigrees from the Sephardic Jewish population in Israel, and American families with panic disorder (19 families) and with SZ. Molecular scanning of candidate genes in unrelated ill patients, and demonstration of more variants in patients than controls, is a powerful alternative strategy to linkage. We now have collected 86 unrelated probands with SZ and 44 with BP for these studies.

A course on systematic diagnosis and interviewing was given for the fifth time this year. This project also includes a prospective study of adolescent and young adult children at high risk for affective disorder by virtue of the fact that they are offspring of bipolar parents.

PROJECT DESCRIPTION

An outpatient clinic for treatment of bipolar manic-depressive (BP) disorder is maintained for the purpose of treating affected persons from multiplex families suitable for genetic studies. We currently treat approximately 50 persons with schizoaffective (SA) or BP disorder. The majority of these persons are from families participating in genetic studies. The majority of BP families that have cooperated in our molecular genetic studies are represented in our clinic population. This ongoing relationship with family members ensures that new diagnostic information will come to our attention promptly.

Pedigree collections: We are participating in the NIMH collaborative study on "Diagnostic Centers for Psychiatric Linkage Studies", and performing other pedigree collections.

We are also collecting samples of unrelated ill persons for association studies based on molecular scanning of candidate genes.

This project includes a prospective study of adolescent and young adult children at high risk for affective disorder by virtue of the fact that they are offspring of bipolar parents. It is expected that 30% of these children will eventually develop affective disorder.

A course on systematic diagnosis and interviewing was given for the fifth time this year, with 25 participants from within NIH/ADAMHA.

RESULTS

I. NIMH collaborative study

The major efforts on this collaboration in its first two years were directed at designing interview instruments and developing protocols for ascertainment and diagnosis. A reliability study of the Diagnostic Instrument for Genetic Studies (DIGS) has been performed and submitted for publication. The Family Interview for Genetic Studies (FIGS), for which this Branch was responsible, has been finalized, based on the family history instrument previously developed here, for which a reliability study was previously published (Arch. Gen. Psychiat. 41: 173-180, 1984). The reliability of the revised instrument will be tested against interview and final diagnoses as the study progresses.

We are also collecting pedigrees from Mediterranean families with BP illness. The study of Sardinians in Sardinia was completed, and Sephardic Jews are still being collected in Israel, to test hypotheses of Xq28 linkage of BP illness in those populations. The hypothesis was rejected in the Sardinian families.

II. Schizophrenia and panic disorder

We have now identified 45 pedigrees of schizophrenic probands, consisting of 396 persons, of whom 104 are classified as affected (either schizophrenic or schizoaffective diagnoses). Lymphoblastoid cell lines have been established

on 270 of these persons.

Panic disorder: 53 families were ascertained for panic disorder, but only 19 have so far met our criteria for inclusion in the series, and agreed to participate in the study.

III. Association studies

Molecular scanning of candidate genes in unrelated ill patients, and demonstration of more variants in patients than controls, is a powerful alternative strategy to linkage. Up to now, we have collected 41 new patients with SZ and 8 with BP for these studies, giving us a total of 44 unrelated BP patients and 86 unrelated SZ patients.

IV. High-Risk Study

We are conducting a longitudinal study of the well adolescent offspring (age 15-25, N=30) of bipolar parents, compared to the adolescent children (N=56) of normal parents to determine whether biological or psychological variables might predict who will become ill. We are conducting a systematic follow-up of these young adults to provide the answer to this question.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND THE PROGRAM OF THE INSTITUTE

Systematic genomic mapping in pedigrees with illness, using molecular linkage markers, offers one of the major opportunities for discovering single loci contributing to illness in an complex inheritance disorder. The possibility of genetic heterogeneity in BP disorders requires that substantial numbers of moderate sized families be studied to be able to detect linkage when only some fraction of the affected population has illness which is linked to a locus under study. Our BP pedigree series has sufficient statistical power to detect linkage in the presence of substantial heterogeneity. We are also undertaking smaller pedigree collections in two Mediterranean populations, which are reputed to have more genetically homogeneous BP illness.

The identification of a similar series of schizophrenic pedigrees is proving more difficult, due to the reduced probability that an affected subject will marry and have children. While the schizophrenic pedigrees identified so far are not sufficient to undertake a genomic screening, they are suitable to test a specific linkage hypothesis.

The development of a series of moderate-sized multiplex families of panic disorder probands, to be used in a linkage study of that disease, would be a very useful approach to the origins of anxiety disorders.

Association is another genetic approach, which bypasses families entirely, and studies candidate genes in unrelated individuals. A genetic variant which is more frequent in patients than controls, that is, which is associated with illness, may lead to discovery of a functional mutation in that gene (or very close to it) which contributes to illness susceptibility.

PROPOSED COURSE OF STUDY

We plan to continue the process of identifying pedigrees with BP and schizophrenic disorders. We will continue to establish lymphoblastoid cell lines where families have six or more ill persons, using disease classifications described above.

We plan to continue the study of multiplex panic disorder families with the goal of collecting a series of moderate-sized pedigrees, such as we have established for BP disease. This is a multi-year effort. If our experience establishing a BP pedigree series is any guideline, then we estimate that an additional 5 years will be required to develop a comparable resource for the genetics of panic disorder.

We are also establishing a collection of genomic DNA from a large series of unrelated patients with BP and SZ disorders, and controls, for association studies.

Finally, we plan to continue the follow-up of the high-risk young adults who have not yet become ill. This will allow us to test hypotheses regarding psychological and biologic variables and risk for affective disorder.

PUBLICATIONS

Gershon ES, Berrettini WH, Robb A, Martinez M, Goldin LR. A non-Kraepelinian continuity of affective disorders and schizophrenia is suggested by twin and family studies. In: Racagni G, Brunello N, Fukuda T, eds. Biological Psychiatry. New York: Elsevier Science Publishing Company, 1991; 510-12.

Gershon ES, Gillin JC. Viewpoint: what the intramural and extramural systems of federally funded biomedical research can learn from each other. J NIH Res 1992;4:20-8.

Gershon ES, Reider R. Disorders of the mind and brain: schizophrenia and mood disorders. Scientific American, in press.

Gershon ES, Goldin LR, Martinez M, Hoehe M. Detecting discrete genes for susceptibility to manic-depressive or schizophrenia. In: Gershon ES, Cloninger CR, Barrett JE, eds. Proceedings of the 1992 Annual Meeting of the American Psychopathological Association, also to be published as Genetic Approaches to Mental Disorders. Washington, DC: American Psychiatric Press, in press.

Gershon ES, Cloninger CR, Barrett JE. eds. Proceedings of the 1992 Annual Meeting of the American Psychopathological Association, also to be published as Genetic Approaches to Mental Disorders. Washington, DC: American Psychiatric Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 MH 02237-08 CNG
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mapping of Psychiatric Disease & Neurotransmission Related Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	S.D. Detera-Wadleigh	Chemist CNG, NIMH
Others:	S.E. Taymans	IRTA Predoctoral Fellow DEB, NICHD
	M. Karl	Visiting Fellow DEB, NICHD
	G.P. Chrousos	Staff Scientist DEB, NICHD
	C. Smith	Staff Fellow LEC, NCI
	G.L. Hager	Section Chief LMV, NCI
	W-T. Hsieh	Senior Staff Fellow CNG, NIMH
COOPERATING UNITS (if any) Dept. of Cellular Pathology, Walter Reed Hospital (T. Fanning); Department of Psychiatry, Jefferson University (W.H. Berrettini); Department of Biochemistry, University of Navarra, Pamplona, Spain (I.J. Encio)		
LAB/BRANCH Clinical Neurogenetics Branch		
SECTION Section on Clinical Genetics		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.5	1.0	4.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Human glucocorticoid receptor (hGR)</u> promoter fragments subcloned into a vector with luciferase reporter gene were transfected into GR-expressing cell lines to investigate <u>basal transcription activity</u> by <u>transient expression assays</u>. A region with the greatest effect on transcription was identified by systematic deletions of promoter segments. Down-regulation by dexamethasone using the promoter constructs tested so far has not shown impressive repression. <u>Gel mobility shift assays</u> reveal that the putative regulatory sequences that have been tested so far bind nuclear factors in a specific manner. </p> <p> Genomic clones encoding the <u>human mineralocorticoid receptor (hMR)</u> were isolated to establish the <u>gene structure</u> of the receptor, determine the exon/intron sequences flanking the splice sites, and that of the promoter. </p> <p> <u>Phylogenetic analysis</u> of the <u>steroid nuclear receptor</u> superfamily reveals at least <u>seven subfamilies</u> and clustered receptors appear to recognize ligands of similar chemical structure. </p> <p> The <u>systematic genomic mapping</u> for a <u>bipolar predisposing locus</u> was continued by genetically typing 20 CNG pedigrees with more markers on additional chromosomes. A total number of 136 loci have been examined and more loci are being analyzed using highly polymorphic markers. </p> <p> The following genes were mapped genetically: <u>cannabinoid receptor gene (CNR)</u> on <u>6q</u>, <u>endothelin-1 gene (EDN1)</u> on <u>6p</u> and <u>phenylethanolamine N-methyltransferase (PNMT)</u> on <u>17q</u>. </p> <p style="margin-top: 20px;"> (Previous Title: Mapping of Psychiatric Disease and Neurotransmission Related Genes-1) </p>		

Principal Investigators: (cont.)

L.R. Goldin	Research Geneticist	CNG, NIMH
M.R. Hoehe	Guest Researcher	CNG, NIMH
E.S. Gershon	Branch Chief	CNG, NIMH
D.Y. Rollins	Biologist	CNG, NIMH
D.S. Muniec	Biologist	CNG, NIMH
T.I. Bonner	Staff Scientist	LCB, NIMH

I. Structure, Regulation and Evolution of the Steroid Receptors

A. PROJECT DESCRIPTION

The regulation of gene expression underlies the control of multiple cellular processes that are involved in development, differentiation, growth and homeostasis. One pathway that leads to these profound cellular changes is triggered by lipophilic hormones which includes the steroids, thyroid hormone, vitamins A and D, and ecdysone. The hormone signals are transduced through binding of hormone to intracellular receptors to form a hormone-receptor complex that specifically binds to enhancer-like sequences termed hormone responsive elements (HRE) on target genes. This binding induces either repression or activation of transcription, thus these receptors are referred to as ligand-activated transcription factors and they potentially regulate the activity of an enormous number of genes, some of which encode transcription factors, in effect establishing a complex hierarchy of gene networks.

To understand the control of various steps involved in cellular processes it is important to unravel the mechanisms involved in the regulation of gene components of signal transduction pathway. In the case of the steroid signaling pathway, studies on regulation of receptor genes have been lacking. One of our major research interests focuses on the study of determinants of gene repression and activation as well as tissue-specific expression. We have chosen the human glucocorticoid receptor (hGR) gene to examine various aspects that control gene repression since this receptor is known to be down-regulated by its own ligand. In addition, the activation of this receptor needs to be examined since it is poorly understood. The question of tissue-specific expression will be investigated using the human mineralocorticoid receptor (hMR) since the tissue distribution of this receptor is highly specific.

B. RESULTS

1. Promoter-mediated Transcriptional Regulation of the Human Glucocorticoid Receptor Gene [S.D. Detera-Wadleigh, I. Encio, C. Smith and G. Hager (NCI)]

As an initial approach to determining the cis- and trans-acting factors that contribute to the regulation of hGR, a 5 kb 5' end fragment of the gene containing exon 1 and the putative promoter was sequenced. We have found DNA sequences that are candidate sites for transcription regulatory proteins, some of which were not previously described. Two strategies are currently being used in order to determine the transcriptional activity of various portions of

the promoter: a) transient expression assays which involves transfection of promoter segments subcloned into a vector with a luciferase reporter gene; and b) gel mobility shift assays which permit evaluation of the ability of putative control elements in recognizing nuclear proteins. The basal transcription activity of the promoter constructs is compared to that obtained in the presence of dexamethasone to characterize the sequences mediating gene repression. Our findings suggest that a major part of the promoter activity is contributed by a portion of about 1 kb that contains multiple Spl and other potential regulatory sites. The putative Apl, half GREs (glucocorticoid responsive elements) and negative GRE sequences, all bound specifically to nuclear factors. These experiments have started to reveal mechanisms by which hGR gene is regulated. The minimal sequences that are essential in promoter activity are being determined both by systematic deletions and site-directed mutagenesis.

2. Genomic Structure and Tissue-Specific Regulation of the Human Mineralocorticoid Receptor [S. Taymans, M. Karl, G. Chrousos (NICHD), and S.D. Detera-Wadleigh]

The importance of hMR in the maintenance of salt and water balance is well known because of its specific binding to aldosterone, thus the expression of the MR gene in kidney and gut. MR is also expressed in substantial amounts in the hippocampus but its role in brain function is not well understood. It is known that hMR in the brain binds with greater affinity than hGR to cortisol. Additionally, some studies have suggested a possible role for these receptors in depression.

The selective tissue distribution of MR provides an excellent system for studying mechanisms that underlie tissue-specific distribution. Our initial approach is to obtain the genomic structure of hMR, isolate the promoter region, conduct transient expression assays using various promoter constructs and gel mobility shift assays. We had predicted previously that the exon/intron organization of the hMR gene would parallel that of hGR. Knowledge of the gene structure also allows identification of evolutionary features which might not otherwise be evident from the expressed sequences alone. In addition, noncoding sequences and splice junctions can be scanned for possible mutations that might correlate with disease.

We have screened a human cosmid library to isolate genomic clones that encode the entire receptor molecule. Genomic clones were individually grown in microtiter plates, transferred to nylon membrane and hybridized with probes taken from sequences of various regions of the receptor. Three overlapping clones have been identified, all containing the putative exon 7 while one contains exon 6 also. Characterization of these clones are underway and rescreening of the library with a mixture of probes to isolate clones encoding the other regions of hMR is being performed.

3. The Phylogeny of the Steroid Receptor Superfamily [T. Fanning (AFIP) and S.D. Detera-Wadleigh]

The evolutionary relationships of receptors in the steroid receptor superfamily was determined by constructing phylogenetic trees using the PAUP

(phylogenetic analysis using parsimony) method. Evolutionary trees were derived by comparing 31 core sequences in the first and second zinc fingers of the DNA binding domains of receptors from different species. The resulting phylogenies revealed that the ancestral gene has diverged into seven major branches represented by the following receptors: retinoic acid (RAR), retinoid X (RXR), glucocorticoid (GR), estrogen (ER), thyroid hormone (TR), vitamin D3 (VDR) and apoA1 regulatory protein-1 (ARP-1). Additional receptors and receptor-like proteins, mostly orphans (receptors with no known ligands), do not cluster with the aforementioned subfamilies. Bootstrap analysis of the consensus sequences for the first and second Zn fingers indicated that the GR and the ER subfamilies share the highest degree of similarity among receptor subgroups. A close relationship of the RXR and ARP-1 subfamilies was also evident. Interestingly, the second Zn fingers of the VDR and GR/ER showed a closer relationship than their first finger motifs. Receptor relatedness, as delineated by coding sequence homologies, correlated with genomic data which showed that variations in the position of the splice site within the DNA binding domain specify distinct branches in the evolutionary tree.

- II. A Systematic Genomic Scan for a Locus Predisposing to Manic-Depression [S.D. Detera-Wadleigh, W-T. Hsieh, W.H. Berrettini (Jefferson University)
L. R. Goldin, M. Hoehe, D.Y. Rollins, D.S. Muniec and E. S. Gershon]

A. PROJECT DESCRIPTION

Manic-depressive illness is a complex disease with a relatively common lifetime prevalence. Family, twin, and adoption studies suggest that a genetic component is involved in the etiology of this disease. The genetics remains poorly understood, and if there is a single disease locus that is detectable, then it might include the confounding influence of incomplete penetrance, possible genetic heterogeneity, uncertain affection status, and possible presence of phenocopies. The lack of a persuasive evidence for an obvious candidate gene and known underlying biochemical abnormality has hampered studies at the molecular level. Attempts have been made to initially search for a genomic region linked to illness but these have resulted in uncertainty because of non-confirmation of positive linkage results that were obtained from a limited number of pedigrees. Most of the genome has not been examined, it is possible that a detectable predisposing locus involved in disease susceptibility in a significant percentage of manic-depressive pedigrees remains to be identified. Our strategy involves a systematic genomic screening for this locus on a panel of up to 20 medium-sized pedigrees. Simulation studies have found that this pedigree series has sufficient power to detect linkage even in the presence of heterogeneity. The existence of appropriately spaced polymorphic markers in a considerable number of chromosomes contributes to the feasibility of the hunt for a disease locus.

B. RESULTS

The systematic bipolar mapping was continued in two different ways: by moving on to other chromosomes and using new, highly polymorphic markers such as microsatellite repeats. So far the following number of loci on different

chromosomes have been examined: 29 on chromosome 2, 14 on chromosome 4, 24 on chromosome 5, 20 on chromosome 7, 11 on chromosome 9, 6 on chromosome 10, 15 on chromosome 11, 3 on chromosome 16, 7 on chromosome 20, 17 on chromosome 3, 10 on chromosome 22 and 3 on chromosome 21. Some positive lod scores have been obtained in a few loci, some of which warrant a more refined and detailed linkage mapping using more informative probes that are within the locus region. Most of the markers that are currently being used are microsatellite repeats and these are multiplexed during gel analysis to achieve greater speed and efficiency.

III. Genetic Mapping of Candidate Genes/Analysis of Candidate Genes

- A. Genetic linkage of the human gene for phenylethanolamine N-methyltransferase (PNMT), the adrenaline-synthesizing enzyme, to DNA markers on chromosome 17q21-q22. [M.R. Hoehe, R. Plaetke, B. Otterud, D. Stauffer, J. Holik, W.F. Byerley, E.E. Baetge, E.S. Gershon, J-M Lalouel and M. Leppert]

Due to the recent progress in the construction of an extended genetic linkage and index map for human chromosome 17 (R. White, P. O'Connell, R. Plaetke, personal communication), we succeeded in determining the genetic location of PNMT, the terminal enzyme of the catecholamine pathway catalyzing the synthesis of epinephrine from norepinephrine. Multipoint linkage analysis placed the PNMT locus in the interval FLB17.1 - CMM86 (D17S74). The human chromosomal region 17q21-q22 identified here to harbour the PNMT gene may be syntenic to the chromosomal region in the stroke-prone spontaneously hypertensive rat (SHR-SP) recently linked to blood-pressure regulation by Hilbert et al. (1991) *Nature* 353: 521-529, and Jacob et al. (1991) *Cell* 67: 213-224. As an increase of PNMT activity has been associated with the development of hypertension in SHR-SP, comparative mapping of this candidate gene is in progress in collaboration with Drs. Eric Lander and Howard Jacob at the Whitehead Institute, Boston, MA.

A role of this enzyme (inherited differences in the activity of which are associated with altered α_2 adrenoceptor sensitivity in an animal model) in the pathogenesis of MDI has been excluded, analyzing its flanking markers pCMM86 (D17S74) and pHHH152 (D17S32) in 21 MDI pedigrees in collaboration with Dr. W.H. Berrettini. The LOD scores as obtained by three-point linkage analysis ranged between -5 and -15.

- B. The human endothelin-1 gene encoding a peptide with potent vasoactive properties maps distal to HLA on chromosome 6p in close linkage to D6S89. [M.R. Hoehe, H. Ehrenreich, B. Otterud, L. Caenazzo, R. Plaetke, H. Zander and M. Leppert]

We determined the precise genetic location of the human endothelin-1 gene (EDN1) encoding a peptide with extremely potent vasoactive properties, apparently involved in a spectrum of diseases affecting the central nervous system, ranging from infectious to traumatic and degenerative disorders. Of particular interest is its prominent role in AIDS encephalopathy. Analyzing the segregation of a four allele EDN1 polymorphism in 40 CEPH families

including 480 individuals, we detected significant linkage of EDN1 to DNA markers spanning the telomeric half of chromosome 6p. EDN1 was closest to the highly polymorphic nucleotide-repeat marker D6S89 at a $\theta = 0.06$ with the highest pairwise LOD score $Z_{\max} = 31.2$. Subsequent multipoint analysis placed EDN1 at 8 centiMorgan (cM) distal to D6S89; EDN1 was flanked at its telomeric site at a 13 cM distance by the gene encoding the subunit of blood clotting factor XIII (F13A1). Furthermore, EDN1 was located at approximately 34-36 cM distal to the HLA region defined by HLA-A, -B, and -DRB1, and 31 cM proximal to the most telomeric marker D6S7. This location of EDN1 on the primary genetic map is strongly supported with odds of $2.7 \times 10^{12}:1$ against the next best alternative. The mapping of EDN1 will particularly assist in locating the gene for autosomal dominant HLA-linked spinocerebellar ataxia (SCA 1).

The EDN1 marker has been analyzed in MDI pedigrees, linkage to the disease has been excluded with LOD scores in the range of -5 to -7.

- C. Genetic mapping of two brain-expressed candidate genes to human chromosome 6. [M.R. Hoehe, M.M. Martinez, B. Otterud, L. Caenazzo, W.S. Modi, H. Ehrenreich, E.S. Gershon and M. Leppert]

The genetic mapping of the 1) human cannabinoid receptor gene (CNR) and the 2) human endothelin-1 gene (EDN1) meant a significant contribution to the construction of the chromosome 6 CEPH consortium map currently in progress (H.Y. Zoghbi, H.T. Orr, H. Blanche, M.R. Hoehe, R. Cottingham, H. Cann, H. Donis-Keller, in preparation). The two genetic maps we constructed for the inclusion of these two candidate genes cover about 91 cM = about 40% of human chromosome 6 in total: 1) CGA/CNR--4.0--D6S27--4.3--D6S26--8.6--B3.6--6.4--D6Z1 (cen). 2) HLA-DRB1--1.1--HLA-B--0.8--HLA-A--6.5-D6S8--19.4--D6S89--8.2--EDN1--13.2--F13A1--18.1--D6S7 (tel) (maximum likelihood estimates of the sex average genetic distances in centiMorgan (cM) given between the loci).

The preliminary CEPH chromosome 6 consortium map will particularly assist in our MDI disease gene mapping effort, and has been communicated by Drs. Hoehe and Berrettini.

D. Linkage studies with the human cannabinoid receptor gene and its flanking markers D6S27 and D6S26 have been completed in the MDI pedigrees. Three-point linkage analyses excluded an involvement of this candidate gene as SML in MDI with LOD scores ranging from -10 to -25. Manuscript in preparation: M.R. Hoehe, L.R. Goldin, M.M. Martinez, L. Caenazzo, D. Muniec, W-T Hsieh, E.S. Gershon (1992) Genetic linkage studies with the human cannabinoid receptor gene in manic-depressive illness.

E. The genetic mapping of four human adrenergic receptor (AR) genes has been completed in collaboration with the Department of Genetics and HHMI, University of Utah, Salt Lake City; Drs. M. Leppert and J-M Lalouel finalized the necessary multipoint linkage analyses, extensive calculations were particularly necessary to obtain conclusive results on chromosome 5. M.R. Hoehe, B. Otterud, W-T Hsieh, D. Stauffer, J. Holik, W.F. Byerley, E.S. Gershon, J-M Lalouel and M. Leppert (1992) Genetic mapping of AR genes in humans. *Nature Genetics*, submitted.

Because recent evidence has moved the proximal boundary of the HD target region back towards D4S10, a reevaluation of the role of the alpha2-C4 AR gene appeared appropriate (Ken Buetow, Ray White, personal communication). An extensive analysis of the AR marker identified by Hoehe et al. (1991), Nucl Acids Research 20: 10148, as well as of an additionally found highly informative marker of this gene (Riess, Hoehe, Regan, Hayden, in preparation) in Huntington's disease gene families including more than 800 individuals is being completed in collaboration with Drs. Michael Hayden and Olaf Riess, Vancouver.

F. The evaluation of these four AR genes and their flanking markers has been completed in our MDI pedigree series, multipoint LOD scores in the range of -5 to -20 were obtained: M.R. Hoehe, W.H. Berrettini, L.R. Goldin, S.D. Detera-Wadleigh, L. Caenazzo, K-U Lentjes, and E.S. Gershon (1992) Molecular genetic studies of alpha and beta AR in manic-depressive illness. Arch Gen Psychiatry, to be submitted.

- G. A two-allele PstI RFLP for the alpha1C AR gene. In our ongoing efforts to map AR genes, we have identified a RFLP of a human alpha1C AR gene [M.R. Hoehe, W.H. Berrettini, D. Schwinn and W-T Hsieh (1992)]

This AR gene marker was subsequently analyzed in 40 CEPH pedigrees, significant linkage with pairwise LOD scores in the range of 12.1 to 3.2 was obtained with seven DNA markers physically assigned to 8p21-p22. The maximum likelihood gene order for the inclusion of this AR gene is presently being determined in collaboration with Dr. Maria Martinez, INSERM, Paris: M.R. Hoehe, W.H. Berrettini, E.J. Kozlow, D.A. Schwinn, W-T Hsieh (1992) Genetic mapping of a human adrenergic receptor gene encoding an alpha1C subtype to DNA markers on chromosome 8p. Genomics, to be submitted.

The analysis of this AR subtype in MDI is at present performed in collaboration with Dr. Berrettini.

Generally, the alleles of all the candidate genes mentioned above have, in addition to linkage analyses, been evaluated for association to MDI in all unrelated affected individuals available from the pedigrees.

H. Also, prior data from neuroendocrinological studies have been intensively reviewed and summarized to the first report of a systematic influence of opiate receptor agonists of plasma catecholamine concentrations in humans. A recent report on the increase of plasma catecholamines in newborn of substance-abusing mothers could point to a physiological significance of our findings. M. Hoehe and T. Duka: Opiates increase plasma catecholamines in humans. Psychoneuroendocrinology (1992) in press.

IV. SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROPOSED COURSE OF STUDY

Knowledge of the mechanisms involved in the regulation of the steroid receptors permits understanding of the one of the many important steps in the cascade of reactions leading to metamorphosis, morphogenesis, differentiation

and developmental progression. These processes are controlled by interacting pathways involving activation and suppression of gene activity. We will continue our experiments on hGR and hMR to identify determinants for gene repression and tissue specific expression. The possible role of these receptors in late onset diseases will be easier to examine since we will have the genomic structure of both receptors.

The predisposing gene for manic-depressive illness continues to escape identification. We will continue the systematic linkage mapping using highly informative markers which are being generated at a fast rate. As better genetic maps become available, we expect to generate a more informative exclusion map.

PUBLICATIONS

Berrettini WH, Detera-Wadleigh SD, Goldin LR, Martinez MM, Hsieh W-T, Hoehe MR, Choi H, Muniec D, Ferraro TN, Guroff JJ, Kazuba D, Harris N, Kron E, Nurnberger JI, Alexander RC, Gershon ES. A systematic search for vulnerability genes in manic-depressive illness. In: Gershon ES, Cloninger CR, Barrett JE, eds. Proceedings of the 1992 Annual Meeting of the American Psychopathological Association also to be published as Genetic Approaches to Mental Disorders, Washington, DC: American Psychiatric Press, in press.

Hoehe MR, Martinez M, Otterud B, Caenazzo L, Modi WS, Ehrenreich H, Gershon ES, White R, Lalouel JM, Leppert M. Genetic mapping of two brain-expressed genes to human chromosome 6. Cytogenet Cell Genet, in press.

Hoehe M, Duka T. Opiates increase plasma catecholamines in humans. Psychoneuroendocrinol, in press.

Hoehe MR, Berrettini WH, Schwinn D, Hsieh W-T. A two-allele PstI RFLP for the α 1C adrenergic receptor gene. Hum Mol Genet, in press.

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Detera-Wadleigh SD, Berrettini WH, Goldin LR, Martinez M, Hsieh W-T, Hoehe M, Encio IJ, Coffman D, Rollins DY, Muniec D, Choi H, Guroff J, Wiesch D, Thai N, Gershon ES. A systematic search for a bipolar predisposing locus on chromosome 5. *Neuropsychopharmacol* 1992;6:219-29.

Fong D, Chan MMY, Hsieh W-T, Menninger JC, Ward DC. Confirmation of the chromosomal assignment of the human cathepsin B gene (CTSB) to 8p22. *Hum Genet* 1992;89:10-2.

Grewal RP, Martinez M, Hoehe M, Bonner TI, Gershon ES, Detera-Wadleigh SD. Genetic linkage mapping of the m4 human muscarinic receptor (CHRM4). *Genomics* 1992;13:239-40.

Hoehe MR, Plaetke R, Otterud B, Stauffer D, Holik J, Byerly WF, Baetge EE, Gershon ES, Lalouel J-M, Leppert M. Genetic linkage of the human gene for phenylethanolamine N-methyltransferase (PNMT), the adrenaline-synthesizing enzyme, to DNA markers on chromosome 17q21-q22. *Hum Mol Genet* 1992;1:175-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02463-04 CNG
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Mathematical Issues in Genetic Analysis		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute) PI: L.R. Goldin Research Geneticist CNG, NIMH Others: M.M. Martinez Visiting Fellow CNG, NIMH E.S. Gershon Branch Chief CNG, NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Neurogenetics Branch		
SECTION Section on Clinical Genetics		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 2.5	PROFESSIONAL: 1.25	OTHER: 1.25
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have computed the power to detect linkage under various two-locus models of inheritance in pedigrees and nuclear families and compared <u>methods of linkage analysis</u> . Linkage can generally be detected for epistatic models if the linked locus is dominant and the unlinked locus either dominant or recessive. For an additive model, linkage is more difficult to detect. The power to detect linkage when the true model is two-locus, is not substantially better when the data are analyzed under a two-locus model vs. a single locus model although the estimates of the recombination fraction are better. If a sample of 200 nuclear families are to be collected for a linkage study, the average sibship size should be 4 with at least 2 affected sibs, in order to have enough power if inheritance involves two-locus heterogeneity. Pedigree data on linkage of <u>Alzheimer's disease</u> to <u>chromosome 21</u> and <u>19</u> markers are being analyzed as part of the <u>Genetic Analysis Workshop 8</u> . We are attempting to better localize the gene on chromosome 21 that causes early onset Alzheimer's in some families, by carrying out linkage analysis under more complex models of age-dependent penetrance. <u>Efficiency of linkage calculations</u> for the bipolar mapping project are improving by the development of methods for reducing the number of alleles for marker systems with many alleles and by implementing linkage procedures on high speed computers.		

I. Detection of linkage for two-locus models of inheritance in pedigrees

An important question to consider when attempting to carry out mapping studies in psychiatric disorders is how many genes are involved in determining the trait of interest. We have previously examined the effects of two-locus heterogeneity on the detection of linkage. These studies have been extended to consider other two-locus models of inheritance. We have simulated data based on two-locus epistatic and additive inheritance where one of the loci is linked to a marker locus having four, equally frequent alleles. Parameters for two-locus models were chosen to be compatible with the observed population prevalence ($\approx 7\%$) and recurrence risk in first degree relatives ($\approx 25\%$) for major affective disorders (BP and UP). We have compared the power of linkage detection and the estimates of the recombination fraction for these models when calculating lod scores under single locus vs. two-locus models.

The following table compares the power of linkage detection when data that have been generated under various two-locus models are analyzed under a single locus model, the correct two-locus model, and incorrect two-locus models.

TABLE 1. TWO-LOCUS EPISTATIC MODELS

Comparison of one-locus vs. two-locus linkage analysis

Simulating Model		θ	Analysis Model	Power	Average	Average
Locus A	Locus B				Max. Lod	θ
Dom	Dom	.05	Single Locus*	74%	5.45	.123
			Dom - Dom	84%	6.19	.050
			Dom - Rec	78%	5.90	.079
			Add - (equal)	72%	5.07	.020
Dom	Rec	.05	Single Locus	88%	5.43	.100
			Dom - Dom	94%	6.21	.046
			Dom - Rec	96%	6.60	.057
			Add - (equal)	90%	5.06	.008

Sample size : 20 pedigrees, at least 2 affected/sibship

*Single locus model: Dominant, 50% penetrance

TABLE 2. TWO-LOCUS ADDITIVE MODELS

Comparison of one-locus vs. two-locus linkage analysis

Simulating Model <u>Locus A - Locus B</u>	<u>θ</u>	<u>Analysis Model</u>	<u>Power</u>	<u>Average Max. Lod</u>	<u>Average θ</u>
Additive (equal effects)	.05	Single Locus*	32%	2.43	.214
		Dom - Dom	32%	2.55	.164
		Dom - Rec	32%	2.47	.183
		<u>Add - (equal)</u>	<u>38%</u>	<u>2.73</u>	<u>.058</u>
Additive (unequal effects)**	.01	Single Locus	0%	.15	
		<u>Add - unequal</u>	<u>0%</u>	<u>.29</u>	

Sample size : 20 pedigrees, at least 2 affected/sibship

* Single locus model: Dominant, 50% penetrance

** linked locus, A, has smaller effect than B

These results indicate that power of linkage detection is not substantially improved when analyzing the data under a two-locus model compared to a single locus model with reduced penetrance even though the two-locus model is the correct model for the data. The power is highest assuming the correct two-locus model but other reasonable two-locus models have power similar to the one-locus model. However, the estimate of θ is better under the two-locus model. Nonetheless, single locus analyses appear to be a good screening method for detecting linkage. If there is some independent evidence that two-loci are involved in a trait or if there were a marker locus for each disease locus, then a two-locus analysis may be worth doing.

These analyses also indicate that the power to detect linkage is high for epistatic models, if the linked locus is dominantly inherited. As table 2 indicates, a gene acting additively will be more difficult to detect. If the linked gene has a smaller effect than the unlinked gene, then linkage cannot be detected at all with this sample size.

II. Ascertainment of nuclear families for linkage studies

The optimal size and ascertainment method for nuclear families for linkage studies has been examined in order to advise the NIMH Genetics Initiative bipolar subcommittee.

It is assumed that 200 nuclear families will be sampled with at least two affected sibs per family. We have found that if there is extreme heterogeneity (that is, if the proportion of linked families is 25%), then the total sibship size needs to be at least 4 (power of linkage detection is

approximately 70%). If the sibship size is 3, then the power is only 50%. If a two-locus model of inheritance is assumed, then the power depends on the mode of inheritance of the linked locus. If the linked locus is dominant and the unlinked locus is either dominant or recessive then the power to detect linkage is virtually 100%. If the two loci are additive, then the power is at least 80% if the sibship size is 3 or more. It appears that even with this large sample size of nuclear families, the presence of substantial heterogeneity will make linkage difficult to detect.

III. Analysis of linkage in Alzheimer's disease pedigrees for the Genetic Analysis Workshop 8.

Three sets of pedigrees, both early and late age of onset, segregating for Alzheimer's disease have been made available for the Genetic Analysis Workshop 8 in order to analyze potential linkage to chromosome 21 and chromosome 19. We have begun to analyze the set of pedigrees collected in Boston that are predominantly early onset, in which chromosome 21 linkage was previously detected. In the already published studies, the Alzheimer's gene could be located to a region of chromosome 21 but the location was very imprecise. Our plan is to utilize more complex linkage models in order to better locate the gene for early onset Alzheimer's disease.

From preliminary analyses, the lod scores for linkage are higher when only affected individuals are analyzed even when age correction is applied to unaffected individuals. The highest lod scores are at 10% recombination from D21S1/D21S11 (lod = 4.25) and 5% recombination from APP/D21S210 (lod = 2.8). Thus, there is recombination with each of the loci typed. We are currently attempting to carry out multipoint linkage analyses to find the best location. In addition, further analyses will determine if taking into account genetic heterogeneity, the presence of phenocopies (especially for individuals with later onset), or two-locus inheritance, will help to narrow the location of this Alzheimer's disease gene.

IV. Improvement of efficiency for linkage calculations

The speed and accuracy of linkage calculations have been improved by greater automation (creation of input pedigree and parameter files by computer) and by the implementation of the LINKAGE programs on high speed computers.

The laboratories are beginning to type markers with a larger number of alleles. This makes each marker more informative for linkage analysis. However, linkage computations can be either impossible or extremely slow when the marker locus has a large number of alleles. We have been implementing computer programs to reduce the number of alleles found. The simplest method for doing this, which is currently implemented, is to renumber the alleles in each family. This preserves the marker informativeness but does not preserve information on allele frequencies. However, precise values of the allele frequencies are not critical when the system is highly polymorphic because parents who have untyped marker genotypes can generally be inferred from their offspring. The next step in reducing alleles which is currently in progress

is to develop a computer program that eliminates some alleles in parents who have married into the pedigree, given that no ambiguity will occur. These allele reductions are important, because even on high speed computers, the time to compute lod scores can be very prohibitively long if there are many loci or loci with large numbers of alleles.

We have also made progress in the speed of linkage calculations by implementing the LINKAGE program on a SUN Sparcstation 2. Computations can be now carried out much faster and some analyses that are not possible to do on a PC machine due to space limitations can now be done on the workstation.

V. Proposed course of study

A. Effects of ascertainment methods for pedigrees on detection of linkage

We plan to continue to focus on how ascertainment of pedigrees effects the power to detect linkage for susceptibility genes for psychiatric disorders given that these genes may be inherited in a complex manner. It is important to determine if selecting pedigrees with a large number of affected individuals causes too much intrafamilial heterogeneity when the inheritance of the disease involves several loci.

B. Power of sib-pair methods for pedigree data for screening a large number of marker loci for linkage

Sib-pair linkage methods allow testing for linkage without requiring assumptions about the mode of inheritance of the disease. We will attempt to determine if calculating linkage statistics in our pedigrees from the sib-pairs is a reasonable way to screen for linkage to a large number of marker loci. Pedigree data can be simulated under several genetic models for the disease and then this method can be compared to the lod score method that we currently use. Previously, we have considered the sib-pair method to have insufficient power for linkage analysis, partly because the method is more sensitive to the polymorphism of the markers than is the lod score method. However, it may now be a good alternative for screening for linkage in our mapping study because the markers now being typed in the laboratory are microsatellites which are more highly polymorphic. In addition, since it is likely that several loci may be involved in the inheritance of psychiatric disorders, it is a problem to specify the model of inheritance for calculating lod scores. The sib-pair method is model free and thus may be most useful as an initial data analysis.

PUBLICATIONS

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Amos CI, Martinez M, Bale SJ. Can a susceptibility locus for schizophrenia be excluded from chromosome 5q11-13? [Letter to the Editor]. Am J Hum Genet 1991;48:1206-8.

Martinez M, Goldin LR. Detection of linkage for heterogenous disorders using multipoint linkage analysis. Am J Hum Genet 1991;49:1300-5.

Berrettini WH, Goldin LR, Martinez MM, Maxwell ME, Smith AL, Guroff JJ, Kazuba DM, Nurberger JI Jr, Hamovit J, Simmons-Alling S, Muniec D, Choi H, York C, Robb AS, Gershon ES. A bipolar pedigree series for genomic mapping of disease genes: diagnostic and analytic considerations. Psychiatr Genet 1991;2:125-60.

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Goldin LR. Mapping of chromosome 21 markers in the Venezuelan pedigree. Cytogenet Cell Genet 1992;59:114-5.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02465-04 CNG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Mapping Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	P.V. Gejman	Visiting Scientist	CNG, NIMH
Others:	E.S. Gershon	Branch Chief	CNG, NIMH
	Q. Cao	Special Volunteer	CNG, NIMH
	C. Aimes	Special Volunteer	CNG, NIMH
	A. Ram	Guest Researcher	CNG, NIMH
	L.R. Goldin	Research Geneticist	CNG, NIMH

COOPERATING UNITS (if any)

Metabolic Diseases Branch, NIDDK (A.M. Spiegel, L. de Marco, G.D. Aurbach, S.J. Marx); Surgery Branch, DCT, NCI (J.A. Norton); Department of Medical Genetics, Yale University School of Medicine (A.E. Bale):

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Section on Clinical Genetics

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.75

PROFESSIONAL:

1.25

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Denaturing gradient gel electrophoresis (DGGE) is being utilized to screen for mutations in candidate disease genes. In psychiatric disorders, we are scanning dopamine receptor genes. In proliferative disorders, GTPase activating protein (GAP) is involved in the regulation of normal ras proteins through its catalytic domain, and plays a role in the signal transduction pathway of some growth factors. We screened several tumor types for mutations in GAP. We have found mutations in the SH2 region of GAP, which regulates signal transduction, in basal cell carcinomas.

A new technology of scanning molecules for mutations spectrophotometrically is being developed.

Linkage analysis of manic depressive illness has been investigated by using a set of 57 microsatellite marker loci. Multiplexing techniques were employed for PCR and electrophoresis. We found isolated LOD scores on chromosome 1 greater than 2 in individual pedigrees, but analysis of the series for linkage with heterogeneity did not give significance. Results of simulations show that when the disease locus is not linked to any of the marker loci, there is still a considerable probability of observing at least one lod score greater than 2.

Principal Investigator: (cont.)

F. Whitsitt	Guest Researcher	CNG, NIMH
M.M. Martinez	Visiting Fellow	CNG, NIMH
D. Pickar	Branch Chief	NSB, NIMH
D. Goldman	Chief	LN, NIAAA

Cooperating Units: (cont.)

West Haven VA Medical Center (J. Gelernter); Department of Molecular Biology, Cetus Corporation (G.A. Martin, F. McCormick); Department of Surgery II, University of Occupational and Environmental Health, Japan (T. Mitsudomi, T. Shirakumasa); Abarbanel Mental Health Center, Israel (P. Sirota); Karolinska Institute, Sweden (E. Friedman)

PROJECT DESCRIPTION:

Our scientific approach is to look for problems in which detection of genotypic variation can lead to a solution. We focus on three such problems: detection of genetic linkage of markers to illness in pedigrees, detection of mutation in candidate genes associated with illness in individual patients, and detection of mutations in proto-oncogenes in tumors.

We use the following methods:

Denaturing Gradient Gel Electrophoresis of enzymatically amplified DNA (PCR) is employed to scan DNA sequences for mutations. A 40 base pair GC-rich high melting domain region is attached to either the 5' or 3' end of each amplified fragments, according to the analysis of the thermolability map of each exon. This allows analysis of the highest melting-point domain of the amplified DNA, which is otherwise lost to analysis. DNA fragments showing aberrant electrophoretic mobility in the denaturing gradient gel are directly sequenced.

Genetic linkage mapping is performed with highly informative DNA microsatellite markers (66% average heterozygosity) on a series of moderately-sized North American manic-depressive pedigrees.

RESULTS:

1. Denaturing Gradient Gel Electrophoresis Applications (DGGE)

Dopamine receptor genes in alcoholism and in schizophrenia: [in collaboration with A. Ram, F. Whitsitt, J. Gelernter (Yale University School of Medicine), D. Goldman (NIAAA) and D. Pickar (NIMH)].

The dopamine D2 receptor gene has been hypothesized to be involved in the etiology of alcoholism and schizophrenia. Conditions of PCR amplification

and DGGE have been found for all the D2 dopamine receptor gene exons. To determine if this gene has a mutation associated with either disease we are currently screening DNA samples from unrelated alcoholics and schizophrenics with DGGE techniques.

GTPase Activating Protein (GAP) Mutations in Human Tumors: [in collaboration with E. Friedman (Karolinska, Sweden), G.A. Martin and F. McCormick, Department of Molecular Biology, Cetus Corporation]

GAP is involved in the down regulation of normal *ras* proteins through its catalytic domain and in the signal transduction pathway of some growth factors. We screened 188 human tumors for inactivating mutations in the catalytic domain and activating mutations in the C terminal SH2 region of GAP. Mutations that dramatically decrease the GTPase activity of the catalytic domain of neurofibromatosis type 1 gene, which shares sequence and probable functional homologies to the GAP catalytic domain, have been found in human tumors. Two closely related regions towards the N terminus of the protein whose function is to bind phosphoproteins and thereby direct protein interactions, are the SH2 (Src Homology 2) regions. The SH2 regions in GAP have been shown to be involved in the cascade of signal transduction induced by activated growth factor receptors. No mutations were in the catalytic domain and three tumor-specific mutations in basal cell carcinomas were detected in a conserved region of the SH2 region. Mutations in the SH2 region of GAP may play a role in the process of tumorigenesis.

Failure of Detection of Mutations in the Catalytic Domain of the GAP Gene in Human Lung Cancer Cell Lines: [in collaboration with T. Mitsudomi, T. Shirakusa (Department of Surgery II, University of Occupational and Environmental Health, Japan) and E. Friedman (Karolinska, Sweden)]

We have not found mutations in the catalytic domain of the GAP gene in human lung cancer cell lines by using DGGE.

2. Microsatellites in Linkage Mapping

Linkage analysis of Manic Depressive Illness (MDI): [in collaboration with Maria Martinez (INSERM, Paris), Eitan Freedman (Karolinska, Sweden), Wade H. Berrettini (Jefferson University), Lynn R. Goldin, Qiuhe Cao, and Elliot S. Gershon]

Multiplexing of PCR reactions was developed and optimized, which is an advance over multiplexing of electrophoresis only. We did not find significant evidence for genetic linkage of any of 57 microsatellites to illness.

An average of 261.2 ± 67.6 individuals were successfully genotyped per microsatellite system; other individuals had technically inadequate results. This average is greater than 80% of the set of family members that had the DNA amplified. Twelve loci had more than 300 individuals genotyped (19 families typed per locus). Typically, we have excluded genetic linkage under homogeneity at most of the loci at a distance of 10 to 20 Cm.

We found isolated LOD scores greater than 2, on chromosome 1, in individual pedigrees. The results of analysis for linkage with heterogeneity at the locus with the most positive LOD scores did not support genetic linkage. Using the SLINK program we ran two thousand replicate simulations of 2 moderate sized families. Results of these simulations show that when the disease locus is not linked to any of the marker loci, there is still a considerable probability (up to 30%) of observing at least one LOD score greater than 2. Significance levels must be considered carefully in systematic linkage studies.

Allelic Loss from Chromosome 11 in Parathyroid Tumors: [in collaboration with E. Friedman (Karolinska, Sweden), A.M. Spiegel, L. de Marco, G.D. Aurbach, S.J. Marx (NIDDK), A.E. Bale (Yale University School of Medicine), and J. Norton (NCI)]

Forty one parathyroid tumors from patients with familial multiple endocrine neoplasia type I (FMENI) and 61 sporadic parathyroid tumors were typed with markers on chromosome 11. FMENI has been previously mapped to 11q13. Microsatellite markers were also in the mapping. To quantitate the efficiency of the reaction and the correct amount of DNA in each reaction, a set of primers from a different chromosome (D19S75) were included in the sample reaction mixture (multiplexing). Alleles in blood were compared with alleles in tumor. Fifty eight percent of the tumors from patients with FMENI showed allelic loss with at least one marker on chromosome 11. Twenty six percent of sporadic parathyroid tumors exhibited allelic loss with at least one marker from chromosome 11. As a result of these findings, the region of deletion is better defined than previously: FMENI gene was located within 7.5cM between PGA centromerically and INT2 telomerically. It is possible that some of the tumors that do not show allelic losses have very small deletions that escaped detection with our set of markers. Alternatively, since parathyroid tumors from patients with MENI that do not display allelic losses from chromosome 11 have a mean weight significantly smaller than parathyroid tumors with demonstrable allelic losses a polyclonal expansion phase prior to the emergence of a monoclonal proliferating cell could not be ruled out.

3. Development of Spectrophotometric System for Mutation Detection

DGGE is a chemical simulation of electrophoresis in a temperature gradient. This method, though powerful, has four disadvantages. First, when a large number of samples are screened together, parallel gradients must be used. Not all the mutations detectable by perpendicular gradients can be detected by parallel gradients. Parallel gradients only detect mutations in the lowest melting domain of the amplified product. Second, GC clamped primers, which modify the melting profile of the target sequences, are expensive, and do not always succeed. Third, making chemical gradients and computing melting profiles requires a degree of expertise which only a few researchers have. Fourth, the length of the fragments which can be analyzed in electrophoresis is restricted.

A better method of detecting DNA "melting" (denaturation) is needed. At a given temperature, the structure of DNA (that is, whether it is melted or

helical) depends on composition (proportion of G/C vs. A/T bases) and sequence. A spectrophotometric measurement of DNA absorbance in solution, as a function of precise measurements of temperature, might serve to detect these changes in absorbance. This would bypass the need for gels and for computer analysis of melting domains.

Several recent developments may make this a practical procedure for analysis of large series of specimens: the recent development of precise temperature controllers for multiple spectrophotometric cuvettes, and PCR. We are currently developing this as a technique which may replace denaturing gradient gels, if successful.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROPOSED COURSE OF STUDY:

Knowledge of mutations causing human diseases will allow a better understanding of the etiology and pathophysiology of psychiatric and non-psychiatric diseases. The use of molecular scanning techniques in the psychiatric population will allow to test the candidate gene hypothesis in a large series of patients. This approach will permit to test if a structural defect in particular candidate genes is involved in the pathophysiology of a psychiatric disease, even in the event that this were true only for a small fraction of the patients.

The use of multiplexing techniques with microsatellite chromosomal markers will accelerate the genetic linkage mapping of manic depressive illness.

PUBLICATIONS:

Barbetti F, Gejman PV, Taylor SI, Raben N, Cama A, Bonora E, Pizzo P, Moghetti P, Muggeo M, Roth J. Detection of mutations in the insulin receptor gene by denaturing gradient gel electrophoresis. Diabetes 1992;41:408-15.

Friedman E, Gordeladze JO, Gejman PV, Murtagh JJ, Gertch DS, Tu T. Hypertrophic cardiomyopathy: failure to demonstrate mutations in exon 13 of the cardiac β myosin heavy-chain gene. Basic Res. Cardiol. 1992;87:106-12.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH-02578 -02 CNG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetic Analysis of Brain Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI: U. Hochgeschwender Senior Staff Fellow CNG, NIMH

Others: W. Schwabe Visiting Fellow CNG, NIMH

T.K. McPhail Biologist CNG, NIMH

M. Brennan Senior Staff Fellow CNG, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Unit on Genomics

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

1.5

OTHER:

0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to study molecular mechanisms of brain development by defining the underlying changes in gene expression. Our model system is the murine cerebellum. By cloning genes whose expression is regulated during cerebellar development, we identify genes potentially involved in specific aspects of this process. To determine conclusively the function of these genes, they will be mutated in embryonic stem cells and the phenotypes of mice carrying the mutant alleles will be assessed.

In a screen of 80,000 cDNA clones from a postnatal day 10 (P10) cerebellum (cbm) library, we identified 6 novel cDNA clones whose expression decreases drastically from embryonic day 18 (E18) to adult. Surprisingly, we identified no clones whose expression was higher at P10 than at E18. These results suggest the coordinate shut-down of a class of developmentally significant genes in the early postnatal period.

The cell types expressing these genes are being investigated by in situ hybridization to sections of developing brain.

We have established the embryonic stem cell - transgenic system in our lab to analyze the effect of mutating these genes. Chimeric mice have been made by injection of embryonic stem cells into blastocysts.

Understanding the processes involved in normal brain development will give us important keys in understanding the relationships between development and mental performance as well as aberrant development and mental illness.

Project Description

In order to analyze experimentally how the mammalian brain develops, we study the development of the murine cerebellum - an experimental system which is very accessible, well-characterized and relatively simple but still representative of the developing mammalian brain.

Our experimental approach to understanding aspects of cerebellar development is based upon the assumption that variations in gene expression both underlie and accompany developmental processes like cell proliferation, migration, morphological differentiation and synaptogenesis. Our research goals are first, to identify genes regulated during cerebellar development; second, to correlate the expression of these genes with specific developmental processes; and third, to move to an understanding of the functions of the gene products.

Major Findings

We have screened 80,000 clones of a developing cerebellum (postnatal day 10, P10cbm) cDNA library with cDNA probes of P10cbm and embryonic day 18 cerebellum (E18cbm). Exposures were evaluated in both directions, i.e. for plaques giving signal with the P10cbm probe, but not with the E18cbm probe and vice versa. Six clones showing differential hybridization in the cDNA screenings were verified as developmentally regulated by Northern blot analysis; all 6 clones were downregulated from E18 to P10 to low adult levels. Analysis of partial sequence revealed no homologies to known sequences.

Our results have several important implications. In a screening of 80,000 clones which detected cDNA clones of high, medium and most of the low abundant mRNAs, only 6 clones were identified as developmentally regulated; none of the 6 clones was isolated twice in these screenings. From this we conclude that developmentally regulated genes are of low abundance and that we can expect to isolate more of these genes in subsequent screenings. All of the clones we identified so far are downregulated between E18 and P10. Previous work has shown that a number of genes expressed in the differentiated cells of the adult are first turned on in the first and second postnatal weeks. Taken together with our results, these suggest that one class of genes expressed in the developing cerebellum is turned off in the early postnatal period and another class of terminal differentiation genes is turned on shortly thereafter.

To determine the spatial pattern of expression within the developing cerebellum, identified clones are then analyzed by *in situ* hybridization. During the last year we have set up cryostat sectioning and *in situ* hybridization in the lab and successfully performed *in situ* experiments with probes for known sequences expressed in the brain.

The next step in the analysis of these developmentally regulated genes is their functional analysis. These genes will be disrupted by homologous recombination in embryonic stem cells and these mutant cells will then be used to generate mutant mice. The effect of these mutations on cerebellum development will then be assessed. We have set up the embryonic stem cell - transgenic mouse system in the laboratory. This involved routine culture of embryonic stem cell lines, injection of embryonic stem cells into blastocysts

and transfer of injected blastocysts. We are routinely getting live, chimeric offspring from these experiments and are ready to make embryonic stem cell lines carrying a gene mutated through homologous recombination.

Significance

The proper construction and wiring of billions of neurons to make a functioning human brain is a formidable task. The first step in unravelling the relationships between development and mental performance and aberrant development and mental illness is to gain knowledge of normal brain development. The insights gained from studying the relatively simple and experimentally tractable murine cerebellum can be extended to our understanding of the more complex structures of the human brain. Elucidating the mechanisms of brain development at the molecular level will lead to new insights on the formation and, by extension, the functioning of brain structures, including those underlying behavior, intelligence and cognition.

Proposed Course

Our findings have two implications for the further course of our investigations: 1) there are more developmentally regulated genes to be identified; and 2) developmentally regulated sequences are among the low to rare abundant class of mRNAs. Therefore, we will generate a subtracted cDNA library of developing cerebellum which will be enriched for low abundant sequences. This small library enriched for the sequences of interest will then be screened with subtracted cDNA probes to identify novel genes regulated during cerebellar development.

Clones identified so far as well as those identified in the more sophisticated subtracted screenings will be analyzed for their spatial pattern of expression by *in situ* hybridization to sections of developing brain.

Clones of genes whose expression is temporally regulated during cerebellar development, spatially restricted to the developing cerebellum and correlated to particular developmental processes, are candidates for mutational analysis involving introduction of a mutated copy of the gene into embryonic stem cells for homologous recombination and generating new strains of mice mutant for this gene. These mutant mice offer the possibility of studying the function of the identified gene through analysis of the developing cerebellum lacking this gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH-02579-02 CNG
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Isolation and Functional Characterization of Yeast Cyclophilin Genes		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)		
PI:	M.B. Brennan	Senior Staff Fellow CNG, NIMH
Others:	E.S. Davis	IRTA Predoctoral Fellow CNG, NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Clinical Neurogenetics Branch		
SECTION Unit on Genomics		
INSTITUTE AND LOCATION NIMH, Bethesda, MD 20892		
TOTAL STAFF YEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The yeast <u>Saccharomyces cerevisiae</u> is an attractive system for the analysis of genes conserved during eukaryotic evolution. We have used this system to determine the function of <u>cyclophilins</u>, a family of highly conserved, ubiquitously expressed proteins which bind the immunosuppressor <u>cyclosporin A</u> with high affinity. We isolated two yeast genes, CPR1 and CPR3, encoding cyclophilins by cross hybridization with a rat cyclophilin gene. Expression of both genes in E. coli demonstrates that they encode CsA binding proteins. We have studied disruption mutants of both genes as well as of another cyclophilin gene, CPR2, and of the FK506 binding protein gene, FPR1. The only phenotype observed was the inability of cpr3 mutants to grow on lactate at 37°C. Recent work suggests that the defect lies in the <u>folding</u> or refolding of this enzyme. We have isolated mutants with dominant suppressors of this phenotype, and have prepared a genomic library from one of these strains to clone the suppressor gene by complementation. </p> <p> To extend such analysis to other <u>conserved genes</u>, we investigated a system for <u>selecting</u> conserved genes in yeast. <u>Recombination</u> in yeast is strongly dependent on homology. Accordingly, we expect that transformation of yeast by a plasmid containing a mammalian gene will result in integration of the plasmid at the homologous, conserved yeast gene. To test this, a plasmid containing a rat cyclophilin gene was used to transform yeast; all transformants had integrated at the homologous CPR3 locus. While the length and homology dependence of this recombination must still be determined, this system offers the prospect of systematically identifying conserved genes by transforming yeast with <u>mammalian cDNA</u> libraries. </p> <p> (Previous Title: Isolation and Functional Characterization of Yeast Cyclophilin Genes) </p>		

Project Description

The goal of this project is to identify genes conserved during eukaryotic evolution and to analyze their functions in the model eukaryote Saccharomyces cerevisiae. The productivity of this approach is demonstrated by our determination of the function of the cyclophilin encoded by the CPR3 locus. To extend this approach we are investigating a selection protocol for the systematic identification of conserved genes by homologous recombination between mammalian cDNAs and conserved yeast genes.

Major Findings

We have previously cloned two yeast cyclophilin genes, CPR1 and CPR3, by cross hybridization with a rat cyclophilin cDNA. Sequence analysis suggested that CPR1 encodes a cytosolic form of cyclophilin, while CPR3 encodes an isoform targeted to the mitochondria. To determine precisely the location of the CPR3 gene product, we have produced peptide specific antisera to the predicted CPR3 gene product. The antisera react specifically with the immunizing peptide by ELISA assay. The antisera are now being tested for specificity in Western blots and in immune precipitations. In addition to the structural homology, we have recently shown that CPR1 and CPR3 expressed in *E. coli* bind cyclosporin A. Disruption of these genes, singly or together, did not affect viability under standard growth condition.

We have extended these results by inclusion of disruption alleles of a third cyclophilin gene, CPR2, and of the FK506 binding protein gene, FPR1. The only phenotype observed after extensive analysis of the disruption mutants was the inability of *cpr3* mutants to grow on lactate at 37°C. Disruption of the other cyclophilin loci and of the FPR1 locus did not affect this phenotype, indicating that the cyclophilin isoforms are not redundant. Further, the *cpr3* mutants showed altered activity of the purine biosynthetic pathways at 35°C: *ade2/cpr3* mutants failed to accumulate the purine intermediate amino imidazole ribonucleotide (AIR). This indicates that disruption of CPR3 has pleiotropic effects on mitochondrial function.

The *cpr3* mutants grow on pyruvate, but not lactate. The enzyme lactate dehydrogenase (ferrocytochrome b_2) encoded by the CYB2 gene catalyzes the conversion of lactate to pyruvate. As the mRNA for this gene is properly induced at 37°C, the post-translational processing or stability of ferrocytochrome b_2 requires the CPR3 gene product at 37°C.

We have isolated revertants of *cpr3* mutant strains capable of growth on lactate at 37°C. The revertants isolated in haploid strains are overwhelmingly recessive. As cloning by complementation is more facile with dominant mutants, we have isolated rare dominant suppressors in mutagenized diploid strains. We have constructed a genomic library from one of the dominant revertant strains in a shuttle plasmid. We are now transforming this library into a *cpr3* mutant and selecting for plasmids capable of complementing the defect for growth on lactate at 37°C.

In view of the success in determining a function for a cyclophilin, we were encouraged to explore ways of identifying other conserved eukaryotic genes for study in yeast. Heretofore, conserved genes were identified by nucleic acid hybridization techniques, either cross hybridization or PCR. Both techniques are sensitive to mismatch in the nucleic acid sequences,

although evolution of conserved sequences selects for high but not absolute sequence homology (owing to third base wobble). We reasoned that recombination might not be as sensitive to mismatches. We tested this by transforming yeast with integrative plasmid carrying a rat cyclophilin gene. All of the resulting transformants had integrated the plasmid at the yeast CPR3 locus by homologous recombination through the rat cyclophilin cDNA sequences, although the genes are only 59% homologous. While the dependence of the integration frequency on the length and extent of homology must be determined, this finding opens the possibility of directly selecting conserved genes by the transformation of yeast with mammalian cDNA libraries in shuttle vectors.

Significance

Cyclosporin A is a major drug in the suppression of transplant rejection, but has significant toxic side effects. Cyclophilins are the targets for cyclosporin A activity. By elucidating the function of cyclophilins it may be possible to modify the activity of the drug or to mitigate its side effects.

The central nervous system is affected by cyclosporin A. Knowing the function of cyclophilins may allow us to interpret the CNS manifestations of cyclosporin toxicity.

The conservation of sequence between genes in yeast and mammals suggests a conservation of function. Further the analysis of function in yeast is facile in comparison with a similar analysis in mammalian cells. Accordingly, the isolation of conserved genes and their analysis in yeast will yield insight in their function in high eukaryotes. In addition, a direct selection for conserved genes will open the analysis into unknown aspects of eukaryotic metabolism. The direct selection for, and subsequent analysis of, conserved genes in yeast will provide entree into novel aspects of eukaryotic cell physiology.

Proposed Course

The analysis of the CPR3 locus will proceed in two directions. First, the study of the biochemical nature of the CPR3 gene product, facilitated by the antisera, will include analysis of its peptidyl prolyl isomerase activity, its intracellular localization, and its associated proteins. Second, the nature of the suppressors will be elucidated by their cloning and subsequent genetic and biochemical analyses.

The direct selection of conserved genes by homologous recombination of conserved genes will proceed first by determination of the length and homology dependence of recombination. Next, we will use mammalian cDNAs cloned in shuttle vectors to identify new conserved genes in yeast.

Publications

Davis ES, Becker A, Heitman J, Hall MN, Brennan MB. A novel yeast cyclosporin gene essential for lactate metabolism at high temperature. Proc Natl Acad Sci USA; in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH-02580-02 CNG

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Integrated Transcriptional Maps of Chromosome 21

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute)

PI:	U. Hochgeschwender	Senior Staff Fellow	CNG, NIMH
	M.B. Brennan	Senior Staff Fellow	CNG, NIMH

Others:	A.S. Robb	Medical Staff Fellow	CNG, NIMH
	B.J. Lawrence	IRTA Summer Fellow	CNG, NIMH
	T.K. McPhail	Biologist	CNG, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Neurogenetics Branch

SECTION

Unit on Genomics

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

2.0

OTHER:

0.25

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The aim of this project is to develop and apply methods for identifying transcribed sequences from large chromosomal regions. These methods can be used both in the isolation of disease genes and in making integrated genetic, physical and transcriptional maps. Our approach is to screen large, arrayed genomic libraries with cDNA probes. Basing the transcriptional map on such reference genomic libraries has a number of advantages: physical mapping based on the same clones obviates the need for mapping each expressed sequence clone separately, and, in contrast to cDNA library approaches, only one reference library is needed for identifying transcribed sequences in any number of tissues and developmental stages. We have concentrated our efforts on human chromosome 21. First, our data on chromosome 21 can be integrated with our previous results on the syntenic mouse chromosome 16. Second, trisomy of chromosome 21 results in pleiotropic developmental and functional defects of Down syndrome. We have used two arrayed genomic libraries from chromosome 21. First, as part of the Joint YAC Screening Effort on Chromosome 21 we have isolated yeast artificial chromosome (YAC) clones for the region of chromosome 21 bands 21q22.2-3 between D21S55 and D21S3. These YAC clones cover 1.4 Mb of the approx. 2 Mb between these markers. The human DNA inserts from these YAC clones were subcloned into a lambda vector and arrayed. The second library is the chromosome 21 cosmid reference library from Hans Lehrach (ICRF, London). Screening the reference library with cDNA probes from cerebral cortex and from a T-cell line has identified 886 expressed sequences. We are now identifying exon sequences by cDNA screening of subcloned fragments and by exon trapping protocols.

(Previous Title: An Integrated Transcriptional Map of Chromosome 21)

Project Description

The systematic identification of transcribed sequences from large chromosomal regions is necessary for integrated chromosomal maps in which the transcribed sequences are positioned on the physical and genetic maps. We have developed direct cDNA screening of large, arrayed genomic libraries as a method for identifying transcribed sequences. We are refining this methodology and applying it to human chromosome 21, both to study the organization of the genome and to identify genes involved in Down syndrome.

Major Findings

In order to produce a detailed physical and transcriptional map in the region of chromosome 21q22.2-3 between D21S55 and D21S3, we isolated overlapping human yeast artificial chromosome (YAC) clones across this region. (This region is a large portion of the Down syndrome consensus region, DSCR; partial trisomies containing this region give rise to many of the Down syndrome phenotypes.) This was carried out as part of the Joint YAC Screening Effort on Chromosome 21. To date, we have 7 YAC clones spanning 1.4 Mb of the approx. 2Mb between ETS and ERG. DNA from the YAC containing yeast strains was subcloned, the human DNA containing clones identified and arrayed in microtiter trays.

For a more comprehensive picture of transcription on chromosome 21, we have used the chromosome 21 cosmid reference library from Hans Lehrach (ICRF, London). In screens of 13,824 cosmid clones, representing approximately 7-fold coverage of chromosome 21, we have identified 684 clones containing sequences expressed in adult cerebral cortex and 681 containing sequences expressed in the human T-cell line Molt-4. Of these 479 are expressed by both tissues, 205 are expressed only in cerebral cortex, and 202 are expressed only in the T-cell line.

Screenings have also been carried out on the arrayed cosmid library of chromosome Xq28 of A. Poustka (German Cancer Research Center, Heidelberg). This library consists of 1536 clones. In screens with cDNA probes from cerebral cortex, 80 clones containing expressed sequences were identified. A number of the clones identified in these screens are known to contain genes expressed in human cerebral cortex.

In order to characterize the mRNAs encoded by genomic sequences from chromosome 21 and Xq28, the clones are used to probe Northern blots. Exon sequences have been identified by an exon trapping protocol and by screening small subfragments with cDNA probes.

Our previous results showed that the sensitivity of the cDNA probes can be increased by decreasing the sequence complexity of the template mRNA. In collaboration with Mathias Hediger (Harvard Medical School, Boston), we have isolated size fractions of human T-cell mRNA by electrophoresis in a bull's eye type gel apparatus. These are being used as template for more sensitive cDNA probes.

Significance to Biomedical Research and the Program of the Institute

There are two aspects to the significance of this project. First, it will provide basic information on the structural and functional organization of mammalian chromosomes. This will include data on the numbers of genes in a variety of tissues, their patterns of expression, and their organization on chromosomes. In addition, comparison of systemic regions of mouse chromosome 16 and human chromosome 21 will provide data on the evolution of mammalian chromosomes, especially the degree of conservation in sequence organization.

Second, the methods developed for identifying transcribed sequences for large chromosomal regions will be useful in searches for disease genes known to map in specific physical or genetic intervals. In the case of the chromosome 21 library screenings, genes of possible significance to Down syndrome will be identified.

Proposed Course

The cloning of the region from D21S55 to D21S3 will be completed and the expressed sequences from this region identified by cDNA screenings. Further the homologous sequences from mouse will be isolated in order to compare the organization of the syntenic region of these two chromosomes.

Analysis of the expressed sequence clones from the chromosome 21 reference library will be analyzed for their possible involvement in Down syndrome. The sequences will be placed on the physical map and sequence of exonic regions will be determined. Further screens of the chromosome 21 reference library with cDNA probes from other tissues and from size fractionated mRNA will be used to extend our data on the transcription patterns of genes on chromosome 21.

Publications

Hochgeschwender U. Towards a transcriptional map of the human genome, Trends Genet 1992;8:41-4.

Gardiner K, Brennan MB. Meeting report: first international workshop on the identification of transcribed sequences, Hum Genome News 1992;3:7-9.

Brennan MB, Avdalovic N. A strategy for making a physical map of the human genome, Period Biol 1991;93:583-590.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02626-01 NS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurotoxicity and Neuroprotection		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	S. M. Paul	Chief, Section on Molecular Pharmacology, NSB, NIMH
Others:	F. Finiels-Marlier	Visiting Fellow NSB, NIMH
	A. Marini	Senior Staff Fellow NSB, NIMH
	M. Weller	Visiting Fellow NSB, NIMH
	B. Ni	Visiting Fellow NSB, NIMH
	P. Williams	Chemist NSB, NIMH
	D. M. Chuang	Chief, Section on Molecular Neurobiology BPB, NIMH
	X.M. Gao	Visiting Fellow BPB, NIMH
COOPERATING UNITS (if any) Section on Molecular Neurobiology, BPB, NIMH		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Molecular Pharmacology		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
4	3	1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This is a relatively new project which has characterized a novel neuroprotective mechanism in cerebellar granule neurons. Primary cultures of rat cerebellar granule cells have been used extensively to characterize the mechanisms underlying the toxicity of various neurotoxins, including the excitotoxin glutamate and the Parkinsonian neurotoxin MPP⁺. These toxins kill cerebellar granule cells via different mechanisms. The former requiring elevations in intracellular calcium and the latter requiring intracellular accumulation of toxin. Recently we have found that preincubation of cerebellar granule cells with very low concentrations of NMDA or glutamate (i.e. low micromolar concentrations) which are subtoxic, induces a neuroprotective state against both MPP⁺ and glutamate toxicity. This neuroprotective state requires several hours to fully develop and is mediated by the NMDA subtype of glutamate receptor. Moreover, NMDA receptor-mediated neuroprotection in cerebellar granule cells requires new RNA and protein synthesis since the neuroprotective state is prevented by coincubation with either cycloheximide or actinomycin-D. These data suggest the presence of a receptor mediated and transcriptionally-activated neuroprotective program present in cerebellar granule cells. We have characterized this state both with respect to its kinetics and generalizability to other toxins. We are now attempting to define the exact genes responsible for the induction of this novel neuroprotective state. </p>		

PROJECT DESCRIPTION

Over the years a variety of neurotoxins have been identified and their neurotoxic actions characterized. Several lines of evidence suggest that both endogenous and exogenous neurotoxins may be involved in a number of neurodegenerative disorders. In fact the excitatory amino acid glutamate is toxic to many neurons upon prolonged exposure with relatively high concentrations. This neurotoxicity, termed excitotoxicity, has been postulated to underlie the neuronal damage induced by ischemic and traumatic damage to the CNS. Moreover, some have hypothesized that excitotoxicity may also underlie the neurodegeneration observed in both Huntington's and Alzheimer's disease. Consequently, many laboratories are not characterizing the cellular mechanisms underlying excitotoxicity and developing compounds which can block the development of the delayed-type neurotoxicity observed after prolonged glutamate exposure both *in vivo* and *in vitro*. Recent experiments in our laboratory suggest that preexposure of cerebellar granule cells to low concentration of NMDA or glutamate induces a neuroprotective state against both the excitotoxin glutamate (acting via NMDA receptors) and MPP⁺ which kills neurons in an NMDA receptor-independent mechanism. The neuroprotective state induced by NMDA requires new RNA and protein synthesis and therefore most likely results from the expression of a neuroprotective protein. Given that this neuroprotective state extends to two mechanistically unrelated neurotoxins we believe that understanding the mechanisms responsible for this neuroprotection may result in a better understanding of both the mechanisms underlying the toxic effects of these agents and could lead to the development of a novel neuroprotective agent. Using a variety of methods, such as, two-dimensional gel electrophoresis and subtractive hybridization we are attempting to characterize the genes whose expression is induced following NMDA exposure of cerebellar granule cells in culture. We are also attempting to characterize the signal transduction pathway(s) responsible for this transcriptional activation, as well as to identify the possible neuroprotective protein(s) itself.

MAJOR FINDINGS

To date, we have characterized a robust neuroprotective state induced by low concentrations of NMDA in cerebellar granule cells. As little as 1 μ M NMDA can induce the neuroprotective state against MPP⁺ toxicity. Moreover, NMDA pretreatment can shift the concentration-response curve for glutamate toxicity from an EC₅₀ of approximately 25 μ M to greater than 10 mM! As mentioned above, the neuroprotective state induced by NMDA is time- and concentration-dependent and is blocked by preincubation with cycloheximide and actinomycin D. The NMDA induced neuroprotective state is not observed in primary cultures of cortical or hippocampal neurons from the same species and 3-acetylpyridine toxicity of cerebellar granule cells is not blocked by NMDA preincubation. Preliminary data suggests that extracellular calcium is required for the neuroprotective state and we are currently attempting to define the mechanisms by which calcium activates gene transcription in these cells.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE INSTITUTE

Glutamate is the major excitatory neurotransmitter within the central nervous system. In addition to its well-described depolarizing effects and neurotransmitter function, glutamate is also involved in many important physiological events related to many aspects of behavior, including learning and memory, cognition, etc.. Prolonged exposure of most neurons to glutamate (particularly at high concentrations) can lead to excitotoxic death. The exact mechanisms responsible for excitotoxicity are not fully understood. Given the postulated role of excitotoxicity in the pathogenesis of a variety of different neurodegenerative and psychiatric diseases, the mechanisms underlying the toxic actions of glutamate and now its neuroprotective actions (described for the first time in our laboratory) could be important in understanding the pathogenesis of a variety of different disorders. Moreover, glutamate-induced transcription of neuronal genes may play an important role in a variety of physiological events such as memory and learning. Therefore, characterization of the genes induced by glutamate could be of importance in understanding both the neurotrophic and neuroprotective effects of this neurotransmitter.

PROPOSED COURSE

We will continue to characterize the neuroprotective state induced by NMDA/glutamate receptor activation with respect to other neurotoxins, as well as to ischemic injury. Using two dimensional electrophoresis and subtractive hybridization we hope to identify proteins and genes which are expressed in response to glutamate/NMDA exposure. Hopefully, we will be able to clone these genes, determine the proteins they encode, and study the role of these proteins in both neurotoxicity and neuroprotection.

PUBLICATIONS

Marini AM, Paul SM: N-Methyl-D-aspartate receptor-mediated neuroprotection in cerebellar granule cells requires new RNA and protein synthesis. Proc Natl Acad Sci U S A 89:6555-6559, 1992.

Chuang D-M, Xiao-Ming G, Paul SM: N-methyl-D-aspartate exposure blocks glutamate toxicity in cultured cerebellar granule cells. Mol Pharmacol, 42:210-216, 1992.

Leppin C, Finiels-Marlier F, Crawley JN, Montpied P, Paul SM: Failure of a protein synthesis inhibitor to modify glutamate receptor mediated neurotoxicity in vivo. Brain Res 581:168-170, 1992.

Finiels-Marlier F, Marini AM, Williams P, Paul SM: The N-Methyl-D-aspartate antagonist MK-801 fails to protect dopaminergic neurons from 1-methyl-4-phenylpyridinium toxicity in vitro, in preparation.

Weller M, Marini AM, Paul SM: Niacinamide blocks 3-acetylpyridine toxicity of cerebellar granule cells in vitro. Brain Research, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02627-01 NS																																
PERIOD COVERED October 1, 1991 to September 30, 1992																																		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neuroactive Steroids																																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 60%;">S.M. Paul</td> <td style="width: 25%;">Chief, Section on Molecular Pharmacology</td> <td style="width: 10%;">NS, NIMH</td> </tr> <tr> <td>Others:</td> <td>R.Irwin</td> <td>Staff Fellow</td> <td>NS, NIMH</td> </tr> <tr> <td></td> <td>J.N. Crawley</td> <td>Chief, Unit on Behavioral Neuropharmacology</td> <td>NS, NIMH</td> </tr> <tr> <td></td> <td>C. Mathis</td> <td>Visiting Fellow</td> <td>NS, NIMH</td> </tr> <tr> <td></td> <td>M. Rogawski</td> <td>Chief, Neuronal Excitability Section</td> <td>NINDS</td> </tr> <tr> <td></td> <td>R. Purdy</td> <td>Scientist</td> <td></td> </tr> <tr> <td></td> <td>D. Farb</td> <td>Professor and Chairman Boston University</td> <td></td> </tr> <tr> <td></td> <td></td> <td>School of Medicine</td> <td></td> </tr> </table>			PI:	S.M. Paul	Chief, Section on Molecular Pharmacology	NS, NIMH	Others:	R.Irwin	Staff Fellow	NS, NIMH		J.N. Crawley	Chief, Unit on Behavioral Neuropharmacology	NS, NIMH		C. Mathis	Visiting Fellow	NS, NIMH		M. Rogawski	Chief, Neuronal Excitability Section	NINDS		R. Purdy	Scientist			D. Farb	Professor and Chairman Boston University				School of Medicine	
PI:	S.M. Paul	Chief, Section on Molecular Pharmacology	NS, NIMH																															
Others:	R.Irwin	Staff Fellow	NS, NIMH																															
	J.N. Crawley	Chief, Unit on Behavioral Neuropharmacology	NS, NIMH																															
	C. Mathis	Visiting Fellow	NS, NIMH																															
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	R. Purdy	Scientist																																
	D. Farb	Professor and Chairman Boston University																																
		School of Medicine																																
COOPERATING UNITS (if any) Neuronal Excitability Section, NINDS; Institute of Health Sciences, St. Luke's Roosevelt Hospital Center, NY, NY; Southwest Foundation for Biomedical Research, San Antonio, TX; Dept of Pharmacology, Boston University School of Medicine.																																		
LAB/BRANCH Clinical Neuroscience Branch																																		
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INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892																																		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:																																
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CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																																		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Neuroactive steroids are natural or synthetic steroids with well described actions in altering neuronal membrane excitability, irrespective of whether they are synthesized in the brain. Neurosteroids, many of which appear to be neuroactive, are steroids that have been shown to be synthesized de novo in the central nervous system. Several years ago our laboratory was the first to demonstrate that the progesterone metabolite 3 α -hydroxy-5 α -pregnane 20-on (allopregnanolone) and the deoxycorticosterone metabolite 3 α -21-dihydroxy-5 α -pregnane 20-on (allotetrahydroDOC) are among the most potent ligands of central GABA _A receptors. This work, which is an outgrowth of our work on GABA _A receptors, has led to the concept that certain steroid hormones can rapidly alter the excitability of neuronal membranes by novel nongenomic mechanisms. In fact, for allopregnanolone and allotetrahydroDOC the mechanisms of their potent inhibitory actions (which results in anticonvulsant, hypnotic, and anesthetic effects in animals and man) is most likely due to their ability to bind to GABA _A receptors and selectively and potently modulate (i.e. enhance) GABA-activated chloride ion conductance. This work has led to the detailed characterization of the interactions of steroids with GABA _A receptors and now with several other members of both ligand-gated and voltage-dependent ion channel receptor families. In fact, recent work has suggested that steroids that have rapid excitatory actions on the central nervous system may do so by enhancing the excitatory amino acid neurotransmitter glutamate, acting at specific glutamate receptor subtypes. These findings, coupled with recent work suggesting that steroids can be synthesized de novo within the central nervous system suggest that neuroactive neurosteroids may, in fact, be important (and as yet unappreciated) modulators of neurotransmitter function.																																		

OTHERS:

S.-Z. Lin	Special Volunteer	NS, NIM
S. Lieberman	President, Institute of Health Sciences	
V.D.K. Prasad	Associate Research Scientist, Institute of Health Sciences	

PROJECT DESCRIPTIONOBJECTIVES

1. To further characterize the mechanism(s) of action of neuroactive steroids, specifically those that modulate known ligand-gated ion channels, including those of the GABA and glutamate receptor super-families.
2. To characterize the structural features responsible for steroid activity at these receptors
3. To measure specific neuroactive steroids in the brain using specific radioimmunoassays.
4. To establish the source of these neuroactive steroids within the central nervous system, i.e. are they produced peripherally or centrally.
5. To characterize factors responsible for modulation of neurosteroid concentrations within the central nervous system.
6. To test the hypothesis that these steroids are involved in important physiological events such as modulation of neuronal activity during stressful environmental stimuli.

METHODS EMPLOYED:

A variety of neurochemical, electrophysiological and microspectrofluorometric methods are employed. Primary cultures of hippocampal, cortical, and cerebellar granule cell neurons are used as a source of neurons.

MAJOR FINDINGS

We have confirmed and extended our initial observations on the A-ring reduced metabolites of progesterone and deoxycorticosterone and carefully defined the structure-activity relationships required for steroidal modulation of GABA_A receptors. These studies strongly suggest that steroids, such as allopregnanolone and allotetrahydroDOC, bind directly to GABA_A receptors at binding sites that are distinct from those of the barbiturates and benzodiazepines. Low concentrations of steroids (like barbiturates) augment the inhibitory actions of GABA (by enhancing GABA activated chloride conductance). In the absence of GABA, high concentrations of these steroids directly increase the probability of chloride ion channel opening, again, much like the barbiturates. Since the modulation of GABA-activated chloride conductance by steroids is additive with pentobarbital, it appears that steroids act at a novel steroid recognition site associated with GABA_A receptors. More recently, we and others have observed the pregnenolone sulfate, in addition to antagonizing GABA-activated chloride conductants at low μM concentrations, is also a positive allosteric modulator of the NMDA receptor. Low μM concentrations of pregnenolone sulfate markedly enhance NMDA-induced increases in intracellular calcium $[\text{Ca}^{2+}]$ in cultured hippocampal neurons as measured using fura 2 microfluorimetry. Pregnenone sulfate has no effect on basal $[\text{Ca}^{2+}]$; and does not appear to modulate kainate or quis-AMPA induced rises in $[\text{Ca}^{2+}]$; thus the excitatory effects of pregnenolone sulfate, previously

reported in the rat, may be due to an antagonism of GABA-mediated inhibition as well as a potentiation of NMDA/glutamate-induced excitation. Very recent work has also attempted to establish the structure-activity relationships for steroidal-induced "positive" modulation of NMDA receptors. The data suggest that the steroid sites associated with NMDA receptors are different in terms of their pharmacology than those associated with GABA_A receptors. In other experiments specific radioimmunoassays were developed to measure allopregnanolone and allotetrahydroDOC in biological material. We have measured both steroids in the brain and plasma of rats and man and found that stress dramatically increases the circulating level of these steroids as well as the concentrations present in brain. Moreover, for allopregnanolone, adrenalectomy and ovariectomy reduce plasma levels below the detection limit of our assay. However, significant (≥ 3 ng/g) levels of allopregnanolone (but not allotetrahydroDOC) were demonstrated in the brains of adrenalectomized/ovariectomized rats. Taken together, these data suggest the possible *de novo* synthesis of allopregnanolone within the central nervous system. Thus, allopregnanolone may qualify as being a true neurosteroid. The plasma levels of allopregnanolone have also been measured in women, throughout the menstrual cycle and during pregnancy. As expected, allopregnanolone levels mirror those of progesterone itself, both during the menstrual cycle and pregnancy. The levels of allopregnanolone during the third trimester of pregnancy are approximately 30 ng/ml or ≥ 100 nM. These levels are significant, given the electrophysiological data suggesting that levels as low as 10 nM can augment the inhibitory actions of GABA. In related experiments with Ellen Freeman of the University of Pennsylvania, we have measured allopregnanolone levels after progesterone administration to normal women and have found good correlations between allopregnanolone levels and changes in cognitive performance, mood and energy levels. Again, these data suggest that allopregnanolone is a behaviorally-active metabolite of progesterone, under both physiological and pharmacological conditions. Work this coming year will extend these observations and will attempt to define other examples of neuroactive steroids acting at ligand gated or voltage-dependent ion channels. A number of interesting leads in the behavioral and electrophysiological literature will be pursued.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE INSTITUTE

Neuroactive steroids and neurosteroids may constitute an important class of central neuromodulators. These steroids may, under physiological and pharmacological conditions, enhance or inhibit the activity of neurotransmitters acting at specific receptors. Consequently such steroids may be quite important in effecting a whole host of behaviors, both normal and abnormal. Moreover, given that an important class of psychotropic medication (the benzodiazepine anxiolytics) are known to interact with GABA_A receptors in producing anxiolytic and anti-convulsant actions, it is conceivable that new anxiolytic/hypnotic drugs or anti-convulsant medications can be developed which act at the steroid recognition site. Such work is in progress at several pharmaceutical companies. Finally, it is also plausible that a number of postpartal behavioral symptoms could be due to the rapid reduction in allopregnanolone levels observed after parturition. If so, the administration of these steroid metabolites could provide a therapeutic strategy for treating these symptoms.

PROPOSED COURSE

We will continue our efforts to characterize other examples of neuroactive steroids and will focus our attention on neurosteroids not yet studied vis á vis their neuroactive properties. Work on the biosynthesis and metabolism of these steroids in the central nervous system are in progress.

PUBLICATIONS

Paul SM and Purdy RH: Neuroactive Steroids. FASEB J 6:2311-2322, 1992.

McNeil RG, Gee KW, Bolger MB, Lan NC, Wieland S, Belelli D, Purdy RH, Paul SM: Neuroactive steroids that act at GABA_A receptors: Therapeutic implications. Drug News and Perspectives, Vol 5. Raven Press, Ltd., New York, pp 145-152, 1992.

Purdy RH, Moore PH, Jr, Morrow AL, Paul SM: Neurosteroids and GABA_A Receptor Function. Proceedings of 7th Sardinian Conference on Neuroscience, for publication.

Weissman EM, Morrow AL, Purdy RH, Paul SM: GABA receptor-active neurosteroids: A new class of CNS active stress hormone? Fidia Research Foundation Symposium Series, for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02340-07 NS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Gaucher Disease & Neurogenetic Disorders Directed Toward Gene Therapy.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. I. Ginns Chief, Section on Molecular Neurogenetics, NS, NIMH
Others: M. LaMarca Microbiologist NS, NIMH
B. Martin Visiting Scientist NS, NIMH
E. Sidransky Senior Research Associate NS, NIMH
B. Stubblefield Biologist NS, NIMH
S. Winfield Microbiologist NS, NIMH
V. Tybulewicz Research Scientist Mill Hill, MRC, London, UK
M. Tremblay Visiting Associate LMG, NICHD

COOPERATING UNITS (if any)

Laboratory of Molecular Genetics, NICHD (see Z01-HD-00071-19 LMGD), Human Genetics Branch, NICHD; Interinstitute Genetics Program, NIH; Center for Cancer Research, MIT; and Whitehead Institute for Biomedical Research, Cambridge, MA.

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Neurogenetics

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.6

PROFESSIONAL:

0.7

OTHER:

1.9

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Clinical pathological correlations for human genetic disorders affecting the nervous system are important for the successful development of diagnostic techniques and therapeutic strategies. This goal is also facilitated by a comprehensive knowledge of the biochemistry and clinical heterogeneity of these disorders. Gaucher disease, the most common sphingolipidosis, is extremely useful as a model because of the occurrence of both neuronopathic and non-neuronopathic phenotypes. The basis of the broad spectrum of clinical diversity within the major types of the disorder can also be studied. Once the pathophysiologic mechanisms of systemic involvement in this enzyme deficiency disorder are understood and treatable, the therapy of nervous system dysfunction may be more rationally approached. Basic research on glucocerebrosidase, the enzyme deficient in Gaucher disease, has generated a more detailed understanding of the structure, biosynthesis, intracellular routing, and turnover of the enzyme. These studies complement other studies within our branch focusing on the investigation of the potential and efficacy of gene transfer as a therapeutic approach. Targeted homologous recombination in embryonic stem cells is used to develop appropriate transgenic animal models of Gaucher disease and other genetic disorders. Recombinant active enzymes are being produced in the milk of transgenic animals. Recombinant production of human enzymes and activator proteins is directed toward the development of effective replacement and gene transfer therapy.

OTHERS:

W. Eliason	Chemist	NS, NIMH
H. Westphal	Chief	LMG, NICHD
J. Sidbury	Chief, Section on Human Biochem Genetics	LMG, NICHD
T. Grasty	Guest Researcher	NS, NIMH
A. Klineburgess	Guest Researcher	NS, NIMH
C. McKinney	Staff Fellow	NS, NIMH
R. Mulligan	Senior Staff Scientist	Whitehead Institute
M. Galdzicka	Special Volunteer	NS, NIMH
B. Wall	Scientist	BARC
L. Hennighausen	Biologist	LBM, NIDDK

PROJECT DESCRIPTION

Biochemical: In our study of the molecular biology of Gaucher disease we: purify mutant enzymes; characterize the primary amino acid sequences and post-translational processing of the mutant enzymes; correlate the structural mutations of the protein with the observed clinical heterogeneity; study sphingolipid activator protein and other activators; characterize the structure, organization, and regulation of expression of the normal and mutant glucocerebrosidase genes; investigate the production of large quantities of protein using recombinant DNA methodologies; develop diagnostically useful recombinant DNA tests (i.e., RFLPs); develop an animal model of Gaucher disease; and evaluate the potential of gene therapy for Gaucher disease.

Clinical: Using Gaucher disease as a prototype of inherited disorders having both neurologic and non-neurologic phenotypes, clinical evaluations are undertaken in an attempt to correlate the clinical heterogeneity with both biochemical and genetic data. The role of the macrophage in pathogenesis of the numerous clinical manifestations of this disorder is studied. Specific approaches to therapeutic intervention for Gaucher disease focus on the hypothesis that this disorder is a macrophage disorder. The involvement of hematopoietic derived cells in the pathogenesis of this disorder is crucial to the applicability of gene therapy as a potential therapeutic strategy.

METHODS EMPLOYED

(SEE: 1987 Annual Report, Project Number Z01 MH 02340-02 NS, pp 443-445; Project Number Z01 HD 00071-19 LMGD, LMG, NICHD)

MAJOR FINDINGS

1. Using targeted homologous recombination in mouse embryonic stem cells, mice have been produced that have germ-line mutations in the glucocerebrosidase gene. Homozygous affected mice have glucocerebroside storage in macrophages and die shortly after birth.
2. Scale-up production of recombinant normal human glucocerebrosidase is in progress. A CRADA with ENZON Inc., (Plainfield, NJ) has been developed for scale-up production for clinical trials of polyethylene glycol conjugated glucocerebrosidase. A clinical trial should be started during FY93.

3. Recombinant mouse glucocerebrosidase and human sphingolipid activator protein (SAP) continue to be produced using the baculovirus expression system. The protein is used for antibody production and structural studies.
4. Constructs containing mammary gland promoters and glucocerebrosidase DNA have been used to produce active enzyme in milk of lactating mice.
5. Antibodies are developed and used for studying recombinant human and mouse glucocerebrosidase.
6. Carbohydrate analysis and amino acid sequencing is performed on recombinant glucocerebrosidase using protein immobilized on teflon membranes.
7. The functional and pseudogene loci for the normal and mutant glucocerebrosidase genes continue to be studied.
8. Mutations in patient's DNA continue to be identified.
9. The mouse gene for glucocerebrosidase continues to be characterized and sequenced for creation of additional mouse model using targeted recombination and zygote injection.
10. Useful diagnostic tests are developed based on polymerase chain amplification and hybridization of oligonucleotide probes, for the identification of mutations in Gaucher disease.
11. Studies of the correction of the enzyme deficiency in Gaucher patient in culture by retroviral mediated gene transfer using cDNA retroviral constructs continues.
12. The spectrum of symptoms of patients having Gaucher disease was further studied and correlated to genotype.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

Gaucher disease is a prototype disorder for furthering our understanding of the mechanisms responsible for the clinical heterogeneity seen within many of the neurogenetic disorders. It is the most common sphingolipidoses and many patients could benefit from the development of therapy. The techniques and information obtained from the study of the protein processing and gene expression in Gaucher disease should be useful for formulating strategies for understanding the biochemical and genetic bases of other neuropsychiatric disorders.

PROPOSED COURSE

Patients having type 1, 2 or 3 Gaucher disease will be studied to further define the biochemical and genetic mechanisms responsible for the clinical heterogeneity within this disorder. Mice produced by targeted homologous recombination will be used as a model of Gaucher disease. We will continue to study the recombinant production of human proteins in transgenic animals, including mice and pigs. Work will continue toward the beginning of clinical trials of PEG-modified glucocerebrosidase. The involvement of hematopoietic stem cell derived macrophages in the pathogenesis of symptoms makes Gaucher disease an attractive candidate for gene therapy.

PUBLICATIONS

Sidransky E, Frenkel E, Benear JB, Ginns EI: Anemic Gaucher patients with elevated endogenous erythropoietin levels may not respond to recombinant erythropoietin therapy. *Blood* 1992;79:532-533.

Sidransky E, Ginns EI: Erythropoietin levels in Gaucher patients. *Am J Hematol* 1991;40:153-154.

Tybulewicz V, Tremblay ML, LaMarca ME, Stubblefield BK, Winfield S, Zablocka B, Sidransky E, Martin BM, Westphal H, Mulligan RC, Ginns EI: Generation of chimeric mice with glucocerebrosidase gene mutations introduced by targeted homologous recombination in embryonic stem cells to produce a mouse model of Gaucher disease. *Am J Hum Gen* 1991;49:441.

Tybulewicz VLJ, Tremblay ML, LaMarca ME, Willemsen R, Stubblefield BK, Winfield S, Zablocka B, Sidransky E, Martin BM, Huang SP, Mintzer KA, Westphal H, Mulligan RC, Ginns EI: Animal model of Gaucher's disease from targeted disruption of the mouse glucocerebrosidase gene. *Nature* 1992; 357:407-410.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 02341-07 NS
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Correction of Inherited Protein Deficiencies by Gene Therapy		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: E.I. Ginns, Chief, Section on Molecular Neurogenetics, NS, NIMH Others: B. Martin Visiting Scientist NS, NIMH E. Sidransky Senior Research Associate NS, NIMH C. McKinney Senior Staff Fellow NS, NIMH B. Stubblefield Biologist NS, NIMH M. LaMarca Microbiologist NS, NIMH S. Winfield Microbiologist NS, NIMH W. Eliason Chemist NS, NIMH		
COOPERATING UNITS (if any) Section on Preclinical Neuroscience, NPB, NIMH; Center for Cancer Research, MIT and Whitehead Institute for Biomedical Research, Cambridge, MA		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Molecular Neurogenetics		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	1.8	PROFESSIONAL:
	0.7	OTHER:
		1.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The characterization of normal and abnormal proteins in genetic disorders affecting the nervous system permits the isolation of <u>cDNA</u> and <u>genomic DNA</u> that can be used to <u>correct</u> inherited protein deficiencies using <u>gene therapy</u> . Particularly suited for initial attempts at gene therapy are those disorders (such as <u>Gaucher disease</u> , the most common <u>sphingolipidosis</u>) in which the systemic and neurologic manifestations of the disorder are the consequence of abnormalities of <u>bone marrow derived cells</u> , like the <u>macrophage</u> . In these instances the transfer of normal genes to either specific tissue or <u>bone marrow progenitor</u> cells is a rationale therapeutic approach. Using the <u>lysosomal</u> disorder Gaucher disease as a model, we utilize <u>retroviral vectors</u> to transfer and express human glucocerebrosidase in <u>mouse</u> and Gaucher patient cell lines. <u>Receptor mediated gene transfer</u> into tissues is another strategy being investigated. The initial goal of this research is the application of gene therapy to the non-neuronopathic phenotypes. Transgenic animal models are developed using <u>targeted homologous recombination</u> in embryonic stem cells to generate mouse models of human disease. Retroviral mediated transfer of tyrosine hydroxylase for the correction of DOPA deficiency states is also studied. Recombinantly manipulated cells that act as depots of L-DOPA release are being developed for transplantation into the nervous system. When our understanding of the pathogenetic mechanisms of inherited <u>neurologic</u> and <u>psychiatric</u> diseases improves and as <u>retroviral-mediated expression</u> of genes in specific tissues and cells becomes more predictable, we can extend the use of <u>gene therapy</u> to treatment of selected disorders affecting the <u>nervous system</u> .		

OTHERS:

T. Grasty	Guest Researcher	NS, NIMH
W. Freed	Chief, Section on Preclin Neurosc	NP, NIMH
R. Mulligan	Professor	MIT

PROJECT DESCRIPTION

Complementary cDNA and genomic DNA encoding proteins that are important to brain function, are isolated. Gene transfer approaches to the correction of abnormal protein function in and outside of the nervous system is studied. Using both the lysosomal storage disorder Gaucher disease and DOPA deficiency states as prototypes, we are developing gene transfer, receptor-complex targeting of DNA, and cell transplantation, first applied in tissue culture and then in small animals.

METHODS EMPLOYED

(SEE: 1987 Annual Report, Project Number ZO1 MH 02341-02 NS, pp 447-449;
1991 Annual Report, Project Number ZO1 MH 02253-07 NPB)

MAJOR FINDINGS

1. Mapping of both the functional and pseudogene genomic loci for human glucocerebrosidase continues.
2. The flanking regions of the mouse gene for glucocerebrosidase are being isolated and characterized for gene transfer experiments using YAC clones.
3. The correction of enzyme deficiency in Gaucher fibroblasts and mouse cells in tissue culture has been successful. New vectors containing glucocerebrosidase DNA have been constructed and are now being used for transfer of the normal human glucocerebrosidase cDNA to these cells. Expression of the enzyme is being studied.
4. Retroviral mediated transfer of tyrosine hydroxylase to mouse and rat cells has been successful. Studies using rat, mouse and human cells continue. More efficient vectors containing tyrosine hydroxylase cDNA are being constructed.
5. Receptor mediated delivery of DNA to tissue has been successful and continues to be developed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

Our studies should provide results that will permit the design of successful gene therapy for systemic and neurologic disorders.

PROPOSED COURSE

The project initially focuses on the DNA mediated-transfer of normal proteins to rodent and human cell lines in culture. Gene therapy (particularly retroviral-mediated) will be tested next on appropriate animal models and then on human subjects.

PUBLICATIONS

Freed WJ, Geller HM, Poltorak M, Cannon-Spoor HE, et al. Genetically altered and defined cell lines for transplantation in animal models of Parkinson's disease. Prog Brain Res 1990;82:11-21.

Freed WJ, Poltorak M, Takashima H, LaMarca ME, Ginns EI. Brain grafts and Parkinson's disease. J Cell Biochem 1991;45(3):261-267.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02343-07 NS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Genetics of Inherited Neurologic and Psychiatric Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. I. Ginns, Chief, Section on Molecular Neurogenetics, NS, NIMH

Others:	B. Stubblefield	Biologist	NS, NIMH
	E. Sidransky	Senior Research Associate	NS, NIMH
	B. Martin	Visiting Scientist	NS, NIMH
	B. Stubblefield	Biologist	NS, NIMH
	S. Winfield	Microbiologist	NS, NIMH
	J. Hozier	Medical Genetics	FIT

COOPERATING UNITS (if any)

Florida Institute of Technology, Melbourne, FL

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Neurogenetics

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.2

PROFESSIONAL:

0.3

OTHER:

0.9

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We search for mutations responsible for inherited neurological and psychiatric disorders. The phenotypic heterogeneity seen within these inherited disorders can be due to involvement of multiple genes or a consequence of different mutations in a single gene, each affecting protein activity and influencing the processing, compartmentalization and/or stability of the protein. In the case of genetic disorders where a protein abnormality has been identified (such as Gaucher disease), southern analysis and polymerase chain amplification is used to identify mutations that occur in non-neuronopathic and neuronopathic phenotypes. The molecular mechanisms leading to nervous system involvement in these disorders are also studied. The results of this research should provide a molecular basis for diagnosis and formulation of additional therapeutic strategies for these inherited disorders. Genes that may be involved in neuropsychiatric disorders, such as those for neurotransmitter biosynthesis (for example, human tyrosine hydroxylase and tryptophan hydroxylase) and receptors (for example, human GABA receptor) have been isolated. Using DNA markers spaced at 10-20 centiMorgan intervals, we are performing linkage analysis and attempting to identify the gene loci responsible for bipolar illness in an Amish pedigree. (see Project # Z01 MH 02625-01 NS)

OTHERS

None.

PROJECT DESCRIPTION

The observed differences in presentation of neurogenetic disorders can be a consequence of multiple allelic mutations or the involvement of more than a single gene. The understanding of the mechanisms of this phenotypic heterogeneity will be derived from genetic and biochemical analyses. Recombinant DNA techniques are used to isolate and characterize the genes for specific proteins. The study of mutations should elucidate the structural abnormalities and consequences of the abnormal biosynthesis and post-translational processing of the mutant proteins. The identification of RFLPs associated with clinical manifestations is studied using cDNA and genomic DNA probes. The comparison of gene expression for proteins in neural and non-neural tissues should extend our understanding of protein regulation.

METHODS EMPLOYED

SEE: 1987 Annual Report, Project Number ZO1 MH 02343-02 NS, pp 453-455)

MAJOR FINDINGS

1. Complementary DNA and genomic libraries are constructed from neural tissue.
2. Human cDNA and genomic clones encoding active tryptophan hydroxylase are isolated and continue to be characterized.
3. Flanking regions of the functional and pseudogene loci for human glucocerebrosidase are being isolated and studied, utilizing YAC clones containing the region.
4. Chromosome specific microsatellite probes are being used for analysis in an Amish pedigree having a high incidence of bipolar illness. Two-point and multipoint Linkage analysis is being performed.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The description of the molecular basis for neurological and psychiatric disorders should provide a more rational basis for the development of new diagnostic and therapeutic strategies.

PROPOSED COURSE

The project will focus on the biochemistry and genetics of these disorders to obtain a more complete understanding of the mechanisms responsible for the clinical manifestations. Linkage analysis studies will continue to be used to identify the gene locus responsible for bipolar illness in the old order Amish pedigree. This information will be used to develop diagnostic and therapeutic strategies.

PUBLICATIONS

Ginns EI. Gene expression: implications for the study of schizophrenia and other neuropsychiatric disorders. In: Schultz SC, Tamminga CA (eds). Proceeding Int'l Congress on Schizophrenia Research, New York, Raven Press, 1990.

Sidransky E, Stubblefield S, Tsuji S, Ginns EI: Mutation analysis of Gaucher patients with oculomotor abnormalities. Amer J Hum Gen 1991;49:106.

Sidransky E, Tsuji S, Martin BM, Stubblefield B, Ginns EI. DNA mutation analysis of Gaucher patients. Am J Med Gen 1992;41:331-336.

Sidransky E, Tsuji S, Stubblefield B, Currie J, Fitzsimmons E, Ginns EI. Gaucher patients with oculomotor abnormalities do not have a unique genotype. Clin Gen 1992;41:1-5.

Tanaka H, Ishikawa A, Ginns EI, Miyatake T, Tsuji S. Linkage analysis of juvenile parkinsonism to tyrosine hydroxylase gene locus on chromosome 11. Neurology 1991;41(5):719-722.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02344-07 NS

PERIOD COVERED

October 1, 1991 to September 30, 1991

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychiatric Disorders: Protein Structure-Activity Studies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	B. Martin	Visiting Scientist	NS, NIMH
Others:	E. I. Ginns	Chief, Section on Molecular Neurogenetics	NS, NIMH
	W. Eliason	Guest Researcher	NS, NIMH
	L. Possani	Free University	NS, NIMH

COOPERATING UNITS (if any)

Free University of Mexico, Mexico

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Neurogenetics

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

0.7

OTHER:

1.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research is part of an effort to better understand the molecular mechanisms underlying human nervous system development and function, as well as the pathogenesis of certain neurogenetic disorders. Our studies have focused on structural and active site properties of the human neuron specific proteins, lysosomal hydrolases, other proteins (particularly those peptides and proteins that interact with excitable membranes), receptors and venom toxins. Proteins are purified from both human and animal tissues using affinity chromatography, electrophoretic separation, and high performance liquid chromatography. We continue to refine and develop state-of-the-art microsequencing techniques employing both gas-phase and solid-phase protein sequencers. We are presently evaluating the use of capillary zone electrophoresis for the isolation of low amounts of proteins/peptides and DNA from biological materials. Amino acid sequence analysis is carried out on neuronal and non-neuronal proteins and venom toxins. Peptide maps of both normal and mutant proteins are generated using chemical (cyanogen bromide) and enzymatic (trypsin, thermolysin, V8 protease) cleavage. The identification of carbohydrate attachment sites, sulphydryl residues, and intra-chain disulfide residues is used to predict protein structure. Alkylating agents and enzyme inhibitors are used to define active sites. From the primary protein sequence, hydrophobic and hydrophilic domains of the protein are identified.

Information obtained from these protein structure studies permits the design of oligonucleotides and peptides that are synthesized for collaborative research involving antibody production, cDNA cloning, DNA sequence analysis and in vitro mutagenesis, and that are directed toward developing gene therapy.

PROJECT DESCRIPTION

This project focuses on the identification of primary and tertiary structure of the proteins. Once these aspects of protein structure are elucidated, a three-dimensional model can be constructed using the secondary structure data obtained from computer modeling. This information will be used to define the hydrophilic and membrane domains of proteins. Active sites are identified and characterized using sulfhydryl reagents and specific inhibitors and activators. This information is used to design synthetic oligonucleotides and peptides for collaborative research.

METHODS EMPLOYED

(SEE: 1987 Annual Report, Project Number Z01 MH 02344-02 NS, pp 457-458)

MAJOR FINDINGS

1. The amino acid sequence analysis of human and rodent lysosomal enzymes as well as identification of carbohydrate attachment sites and disulfide bridges continues.
2. Biochemical characterization, including partial amino acid sequence of several channel blocking toxins from scorpion, and a thermal regulatory peptide from beaded lizard, as well as cloning of cDNA.
3. Determination of the amino acid sequence of several unusual neurotoxin specific for insects from scorpion.
4. Isolation and amino acid sequence of the first phospholipase found in scorpion venom.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

The neuron specific proteins are used as model systems to investigate the developmentally controlled expression of specific proteins within the nervous system. The lysosomal hydrolases are used as prototypes for understanding the phenotypic heterogeneity disorders affecting the nervous system. Studies of receptors and venom toxins should provide information on the structure of ion channels within the nervous system. Together these studies further our understanding of the structure-function relationships of nervous system proteins.

PROPOSED COURSE

The project will focus on the proteins described above as well as other proteins involved in lysosomal and neuropsychiatric disorders.

PUBLICATIONS

Brzeska H, Martin B, Kulesza-Lipka D, Baines I, Korn ED. Preparation of a phospholipid-insensitive, autophosphorylation-activated catalytic fragment of *acanthamoeba* myosin I heavy chain kinase. *J Biol Chem* 1992;267(7):4949-4956.

Fletcher TS, Kwee IL, Nakada T, Largman C, Martin BM. DNA sequence of the yeast transketolase gene. *Biochemistry* 1992;31(6):1892-1896.

Langford K, Frenzel K, Martin BM, Bernstein KE. The genomic organization of the rat AT₁ angiotensin receptor. *Biochem Biophys Res Commun* 1992;183(3):1025-1032.

Olsen DB, Hepburn TW, Lee S, Martin BM, Mariano PS, Dunaway-Mariano D. Investigation of the substrate binding and catalytic groups of the P-C bond cleaving enzyme, phosphonoacetaldehyde hydrolase. *Arch Biochem Biophys* 1992;296:144-151.

Pace JR, Martin BM, Paul SM, Rogawski MA. High concentrations of neutral amino acids activate NMDA receptor currents in rat hippocampal neurons. *Neurosci Lett* 1992;141:97-100.

Possani LD, Mochca-Morales J, Amezcua J, Martin BM, Prestipino G, Nobile M. Anionic currents of chick sensory neurons are affected by a phospholipase A₂ purified from the venom of the taipan snake. *Biochim Biophys Acta* 1992;1134:210-216.

Zamudio F, Saavedra R, Martin BM, Gurrola-Briones G, Herion P, Possani LD. Amino acid sequence and immunological characterization with monoclonal antibodies of two toxins from the venom of the scorpion *Centruroides noxius* Hoffmann. *Eur J Biochem* 1992;204:281-292.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02625-01 NS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Search for DNA Markers Linked to Manic Depressive Illness in the Old Order Amish.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. I. Ginns Chief, Section on Molecular Neurogenetics, NS, NIMH
S. M. Paul Chief, Section on Molecular Pharmacology, NS, NIMH
Others: J. Egeland Professor Amish Study, University of Miami
D. Pauls Professor Child Study Center, Yale University
E. Sidransky Senior Research Associate NS, NIMH
B. Stubblefield Biologist NS, NIMH
S. Winfield Microbiologist NS, NIMH
J. Shirley Biological technician NS, NIMH

COOPERATING UNITS (if any)

University of Miami; Child Study Center, Yale University

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Neurogenetics

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.1

PROFESSIONAL:

0.6

OTHER:

1.5

CHECK APPROPRIATE BOXES

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are performing a systematic screening of the human genome in order to identify a gene responsible for manic depressive disorder in the Old Order Amish. We are utilizing a large, multigenerational Old Order Amish pedigree with many affected individuals. By using this multigenerational pedigree with a high incidence of a disease manifested by a consistent and definable phenotype, we reduce uncertainties in analysis that could otherwise be introduced as a consequence of genetic heterogeneity, variable mode of inheritance, phenocopies, and/or penetrance. Highly informative markers (RFLP, tandem repeats, dinucleotide repeats, etc.) scattered across the genome are used to obtain genotypings. Due to the uncertainties in ascertaining phenotype and because of the lack of any definitive biological marker for bipolar illness (manic-depressive disorder), the linkage analyses are performed using several clinical hierarchies. Associative analyses are also carried out. Computer simulations using specific models are used to demonstrate the power of analyses. Simulation and data analysis includes several models of inheritance and diagnostic hierarchies, as well as polygenic etiologies. As marker genotypings are accumulated, the diagnostic data is updated, and our collection of cell lines from both normal and affected family member is being expanded. The availability of longitudinal follow-up evaluation for family members and the high frequency of manic depressive illness in the pedigree make the Old Order Amish pedigree a valuable resource in the search for the genetic loci involved in bipolar affective disorder.

OTHERS

J. Weissenbach
T. Keith

Institut Pasteur/Genethon
Collaborative Research

Paris, France
Boston, MA

PROJECT DESCRIPTION

Recently, there has been remarkable progress using positional clonging to identify the chromosomal loci, and in several instances the actual genes, for inherited disorders. However, despite significant effort, the pursuit of cytogenetic abnormalities, association studies or linkage analyses have not yet led to the definitive identification of a locus or gene involved in the etiology of a major psychiatric disorder. We have focused our efforts on the identification of a gene for bipolar illness in a large Old Order Amish pedigree with many affected individuals. We are systematically screening the genome using both restriction length polymorphism (RFLP) and microsatellite markers. By using this large multigenerational pedigree we hope to reduce uncertainties in analysis that could otherwise be introduced as a consequence of genetic heterogeneity, variable mode of inheritance, phenocopies, and/or penetrance. The availability of longitudinal follow-up evaluation for family members and the high frequency of manic depressive illness in the pedigree make the Old Order AMish pedigree a valuable resource in the search for the genetic loci involved in bipolar affective disorder.

METHODS EMPLOYED

We are systematically screening the genome using both RFLP and highly informative microsatellite markers. Initially, markers were spaced at approximately 29 centimorgans. The original collectionm of cell lines from Amish pedigree 110 (NIGMS Human Genetic Mutant Cell Respository, 1987) has been extended. Our analyses are being performed using several different clinical hierarchies. Both linkage and associative analyses are being performed. Computer simulations are used to determine the power of the Amish pedigree to identify a candidate locus for bipolar illness. As we continue to accumulate marker typings, we also update the diagnostic data and expand our collection of cell lines from both normal and affected individuals.

MAJOR FINDINGS

1. The original collection of cell lines from Amish pedigree 110 has been extended to include over 169 individuals.
2. Over 250 markers spaced at approximately 20 centimorgans have been used.
3. Linkage analyses has been carried out using serveral diagnostic schemes.
4. All LOD scores greater than 1.0 are being followed up using neighboring microsatellite markers.

PROPOSED COURSE

We will continue to accumulate marker typings, and we will also update our diagnostic data and expand our collection of cell lines from both normal and affected family members. Analyses will be performed using several clinical hierarchies, with models including different modes of inheritance and variable penetrance. Associative, as well as linkage analyses will be performed. Computer simulations will be used to ascertain the power of the analyses on this pedigree in locating a candidate locus for bipolar illness.

SIGNIFICANCE TO BIOMEDICAL RESEARCH

No gene has yet been identified as responsible for a major psychiatric disorder. The availability of longitudinal follow-up evaluation for family members and the high frequency of manic depressive illness make this Old Order Amish pedigree a valuable resource in the search for the genetic loci involved in bipolar affective disorder. The description of the genetic and molecular basis for manic depressive illness should provide a more rational basis for the development of novel therapies and diagnostic tests.

PUBLICATIONS

Ginns EI, Egeland JA, Allen CR, Pauls DL, Falls K, Keith TP, Paul SM. Update on the search for DNA markers linked to manic depressive illness in the Old Order Amish. J Psychiatr Res 1992. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02181-10 ET

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurobiology of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: D. Pickar, Chief, Section on Clinical Studies ET, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Therapeutics Branch (Formerly: Clinical Neuroscience Branch)

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

PROJECT DISCONTINUED - SEE Z01 MH 02348-01 ET

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02345-05 ET																					
PERIOD COVERED October 1, 1991 to September 30, 1992																							
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) EYE MOVEMENTS IN PSYCHIATRIC AND NEUROLOGIC PATIENTS AND NORMAL VOLUNTEERS																							
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: Robert E. Litman, Senior Staff Fellow, Section on Clinical Studies, ET, NIMH Others: <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">D. Pickar</td> <td style="width: 33%;">Chief</td> <td style="width: 33%;">ET, NIMH</td> </tr> <tr> <td>D.W. Hommer</td> <td>Medical Officer</td> <td>VA</td> </tr> <tr> <td>T. Clem</td> <td>Biomedical Engineer</td> <td>BEI, NIH</td> </tr> <tr> <td>W.W. Hong</td> <td>Staff Fellow</td> <td>ET, NIMH</td> </tr> <tr> <td>F. Torrey</td> <td>Guest Researcher</td> <td>NB, NIMH</td> </tr> <tr> <td>K. Berman</td> <td>Staff Fellow</td> <td>CBD, NIMH</td> </tr> <tr> <td>T. Zeffiro</td> <td>Staff Fellow</td> <td>MN, NINDS</td> </tr> </table>			D. Pickar	Chief	ET, NIMH	D.W. Hommer	Medical Officer	VA	T. Clem	Biomedical Engineer	BEI, NIH	W.W. Hong	Staff Fellow	ET, NIMH	F. Torrey	Guest Researcher	NB, NIMH	K. Berman	Staff Fellow	CBD, NIMH	T. Zeffiro	Staff Fellow	MN, NINDS
D. Pickar	Chief	ET, NIMH																					
D.W. Hommer	Medical Officer	VA																					
T. Clem	Biomedical Engineer	BEI, NIH																					
W.W. Hong	Staff Fellow	ET, NIMH																					
F. Torrey	Guest Researcher	NB, NIMH																					
K. Berman	Staff Fellow	CBD, NIMH																					
T. Zeffiro	Staff Fellow	MN, NINDS																					
COOPERATING UNITS (if any) Department of Psychiatry, VA Medical Center, Seattle, WA; Biomedical Engineering and Instrumentation Branch, NIH; Neuropsychiatry Branch and Clinical Brain Disorders Branch, NIMH; Medical Neurology Branch, NINDS																							
LAB/BRANCH Experimental Therapeutics Branch																							
SECTION Section on Clinical Studies																							
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892																							
TOTAL MAN-YEARS: <div style="text-align: center;">1.5</div>	PROFESSIONAL: <div style="text-align: center;">1.25</div>	OTHER: <div style="text-align: center;">0.25</div>																					
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews																							
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Disorders of eye movement</u> , particularly <u>smooth pursuit eye movement (SPEM)</u> , remain one of the few specific findings in <u>schizophrenia</u> . Despite many studies which find abnormal SPEM in schizophrenia, their precise nature and relationship to the etiology and treatment of schizophrenia remain unclear. This project attempts to better define the pathophysiology of eye movement disorders by studying patients with schizophrenia, other <u>psychiatric disorders</u> and normal controls utilizing a computerized <u>infrared oculographic recording</u> system which allows precise measurement of eye velocity. We have observed differences in <u>SPEM gain</u> and <u>types of saccades</u> in SPEM between schizophrenic patients and controls. We observed <u>intrusive saccades</u> but not <u>corrective saccades</u> to distinguish patients from normals. We observed no effect of <u>typical neuroleptics</u> on these variables, confirming previous findings. However, we find that the atypical neuroleptic, <u>clozapine</u> , significantly reduces SPEM gain and increases catch-up saccades, while intrusive saccades remain stable, regardless of medication or drug-free state. Also, saccades correlate with response to treatment with clozapine. We observed in schizophrenic patients only that SPEM gain is correlated with performance on the <u>Wisconsin Card Sort</u> , a test of <u>frontal lobe function</u> . To further investigate the pathophysiology underlying eye movement dysfunction in schizophrenia. We are analyzing performance on frontal lobe eye movement tasks, as well as changes in regional cerebral blood flow during eye movement tasks utilizing positron emission tomography (PET). Finally, we have examined genetic aspects of SPEM in <u>discordant monozygotic twins</u> with schizophrenia and have found significant differences in SPEM variables between affected and unaffected co-twins.																							

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02346-05 NS

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Immunology of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory and institute affiliation)

PI: M.H. Rapaport, Senior Staff Fellow, NS, DIRP, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

PROJECT DISCONTINUED DUE TO PRINCIPAL INVESTIGATOR'S DEPARTURE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02347-03 ET
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Serotonergic Responsivity in Schizophrenia		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator) (Name, title, laboratory, and institute affiliation) PI: Richard R. Owen, Senior Staff Fellow, Section on Clinical Studies, ET, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Experimental Therapeutics Branch (Formerly: Clinical Neuroscience Branch)		
SECTION Section on Clinical Studies		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="text-align: center; padding: 20px;"> PROJECT DISCONTINUED DUE TO PRINCIPAL INVESTIGATOR'S DEPARTURE </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02348-01 ET
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.) MECHANISM OF ACTION OF ATYPICAL NEUROLEPTICS		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: D. Pickar, Chief, Section on Clinical Studies ET, NIMH Others: R.E. Litman Staff Fellow ET, NIMH T.P. Su Staff Fellow ET, NIMH J.K. Hsiao Staff Fellow ET, NIMH W.W. Hong Staff Fellow ET, NIMH E.M. Weissman Staff Fellow ET, NIMH D.R. Weinberger Chief CBD, NIMH		
COOPERATING UNITS (if any) Clinical Brain Disorders Branch, St. Elizabeths Hospital, NIMH		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Section on Clinical Studies		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">5.0</div>	PROFESSIONAL: <div style="text-align: center;">3.5</div>	OTHER: <div style="text-align: center;">1.5</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The prototype <u>atypical neuroleptic, clozapine</u> , is notable for its enhanced therapeutic in otherwise treatment resistant patients with <u>schizophrenia</u> . We observed in our initial study that over a third of patients respond more favorably to clozapine than to the typical neuroleptic, fluphenazine. We have focused on mechanisms of action of the atypical neuroleptic by addressing clozapine's diverse <u>pharmacologic effects</u> using an array of biological techniques. We observed <u>in vivo</u> "markers" of <u>dopaminergic</u> antagonist effects, <u>serotonergic</u> antagonist effects and enhancement of <u>norepinephrine</u> release. Predictors of clozapine response include high EPS during typical neuroleptic treatment, low plasma HVA during clozapine treatment and enhanced serotonergic responsivity in a drug-free state. In further work, we have applied functional brain imaging, <u>PET</u> and <u>SPECT</u> to discern distinct metabolic effects of clozapine and relationship to <u>D₂ receptor blockade</u> and clinical response. Future plans involve the extension of the data base to include 50 patients and further studies to identify clinical and biological predictors of response.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02349-01 ET

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (50 characters or less. This must fit on one line between the borders.)

IDAZOXAN AUGMENTATION OF PATIENTS WITH SCHIZOPHRENIA

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Co-PI: D. Pickar, Chief, Section on Clinical Studies ET, NIMH

Co-PI: R.E. Litman, Staff Fellow, Section on Clinical Studies, ET, NIMH

Others: T.P. Su Staff Fellow ET, NIMH
J.K. Hsiao Staff Fellow ET, NIMH
W.W. Hong Staff Fellow ET, NIMH
E.M. Weissman Staff Fellow ET, NIMH
W.Z. Potter Chief, Section on Clinical Pharmacology ET, NIMH

COOPERATING UNITS (If any)

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

2.0

PROFESSIONAL:

1.50

OTHER:

0.50

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Idazoxan, a potent α_2 antagonist, has been applied to patients with schizophrenia who are concurrently treated with the typical neuroleptic, fluphenazine. This approach, was based on the α_2 antagonistic properties of the atypical neuroleptic, clozapine. Pilot data have been promising, suggesting that the addition of idazoxan may improve, most notably, negative symptoms in patients with schizophrenia. This important approach may provide further leads into augmenting treatments for schizophrenia.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02350-01 ET
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) FUNCTIONAL NEUROIMAGING TO INVESTIGATE ANTIPSYCHOTIC MECHANISMS OF ACTION		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.K. Hsiao, Staff Fellow, Section on Clinical Studies ET, NIMH Other: D. Pickar Chief ET, NIMH R.E. Litman Staff Fellow ET, NIMH T.P. Su Staff Fellow ET, NIMH W.W. Hong Staff Fellow ET, NIMH E.M. Weissman Staff Fellow ET, NIMH J. Hiday Special Volunteer ET, NIMH P. Herscovitch Chief, PET Imaging Section NM, CC		
COOPERATING UNITS (if any) Nuclear Medicine Department, CC; Laboratory of Cardiac Energetics, NHLBI; Clinical Brain Disorders Branch, NIMH		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Section on Clinical Studies		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">2.5</div>	PROFESSIONAL: <div style="text-align: center;">2.0</div>	OTHER: <div style="text-align: center;">0.5</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) We have combined neuropharmacological and neuroimaging techniques to investigate the <u>mechanism of action</u> of antipsychotics and the pathophysiology of schizophrenia. Traditional investigations of the mechanism of action of antipsychotic drugs have focused on neurochemistry. In recent years, <u>neuroimaging</u> studies have demonstrated the importance of <u>regional brain function</u> in understanding <u>schizophrenia</u> . There have been surprisingly few systematic, controlled investigations into the effects of antipsychotic treatment on regional brain function in man. Over the past year, we have succeeded in demonstrating that the <u>atypical neuroleptic, clozapine</u> , is markedly different from typical neuroleptics in its effects on regional brain glucose metabolism in patients with schizophrenia (using PET-FDG). Furthermore, it appears that clozapine's antipsychotic effects may not be due to changes in any single brain region, but on its effects on a network of interacting cortical and subcortical structures. We have also initiated studies using <u>SPECT</u> and the <u>D₂ receptor ligand, IBZM</u> , to examine the specific role of D ₂ blockade in typical and atypical neuroleptic mechanism of action. Finally, because of the limitations of radioactive tracer techniques (i.e., dosimetry and limited anatomic and temporal resolution), we have begun to use fast <u>MRI</u> to study regional brain function (blood flow and deoxyhemoglobin content). A pilot study has been initiated to compare the effects of <u>D₂ receptor challenge</u> on regional deoxyhemoglobin content during acute and chronic neuroleptic treatment, attempting to visualize the metabolic effects of depolarization blockade.		

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1991, through September 30, 1992

ZOT MH 01850-15 ETB

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of Antidepressants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: William Z. Potter, Chief, Section on Clinical Pharmacology, ETB, NIMH

Others: H. Manji

SCP, ETB, NIMH

K. Dawkins

SCP, ETB, NIMH

R. Risinger

SCP, ETB, NIMH

M. Schmidt

SCP, ETB, NIMH

COOPERATING UNITS (If any)

Laboratory of Clinical Science, NIMH; Laboratory of Clinical Studies, NIAAA
Beersweva Mental Health Center, Israel

LABORATORY

Experimental Therapeutics Branch

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

4.9

PROFESSIONAL:

3.2

OTHER:

1.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Highlights of clinical studies during the last year are:

- a) The first 20 subjects in the USA administered intravenous idazoxan, a selective α_2 antagonist, tolerated the drug well at doses producing selective and marked increases in the release of norepinephrine following an "orthostatic challenge." This provides a new tool for testing α_2 function.
- b) In severely depressed patients ECT selectively alters neuropeptide concentrations in cerebrospinal fluid such that endothelin, a probable modifier of signal transduction through several receptors, is markedly increased, neuropeptide Y is elevated and somatostatin is "normalized" while CRH and neurokinin A are unaffected.
- c) Platelets and leukocytes from lithium-treated manic-depressives show a significant reduction in the immunolabeling of G α , and an increase in pertussis toxin catalyzed [32 P] ADP-ribosylation in cultured lymphoblasts from a separate group of bipolars.
- d) Despite the common classification as benzodiazepine agonists adinazolam and its n-desmethyl metabolite were found to have distinct pharmacodynamic spectrums of activity. Use of pharmacokinetic/pharmacodynamic modelling reveals wide differences in EC_{50} values for each of several effects - sedation ACTH/cortisol suppression, growth hormone release - suggesting that these are mediated by benzodiazepine receptor complexes with different properties.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED

October 1, 1991, through September 30, 1992

Z1 MH 01855-08 ETB

TITLE OF PROJECT (80 characters or less. This must fit on one line between the borders.)

Central Neurochemistry Service

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator. Please list laboratory, and institute affiliation)

PI: W.Z. Potter	Chief	CP, ETB, NIMH
Others: I.N. Mefford	Special Expert	CP, ETB, NIMH
S.P. Markey	Chief	AB, LCS, NIMH
K. Dykstra	Staff Fellow	CE, BEI, NCRR
P.M. Bungay	Chemical Engineer	CE, BEI, NCRR

COOPERATING UNITS (if any)

Section on Analytical Biochemistry, Laboratory Clinical Sciences, NIMH; Chemical Engineering Section, Biomedical Engineering Instrumentation Program, National Center for Research Resources

LAB/BRANCH

Experimental Therapeutics Branch

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

ADAMHA, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.35

PROFESSIONAL:

0.3

OTHER:

8.05

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The central neurochemistry service has provided routine analysis for MHPG, HVA, 5HIAA, 5HT, NE and normetanephrine in plasma. These compounds as well as VMA, DOPAC, epinephrine, dopamine, metanephrine, and 3-methoxytyramine can be measured in urine. In addition, CSE measures of epinephrine, NE, DA, HVA, 5HIAA, MHPG, free and conjugated metanephrine and normetanephrine, and 5HT are possible. Both HPLC with electrochemical detection and GCMS are utilized for quantification. Cumulatively, over 5,000 analyses were provided during the last twelve months.

Further, development of microdialysis methods to interpret what contributes to the overall "extracellular" concentrations in brain of a variety of substances including cyclic AMP derives from application of a theoretical model developed in the Biomedical Engineering Program of the National Center for Research Resources. Diffusion profiles around the probe are being determined for major solutes of interest so that the area which microdialysis samples can be accurately specified. This advance is essential to regional brain studies with in vivo microdialysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01860-06 ET

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

THE ROLE OF EPINEPHRINE IN BRAIN

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and Institute affiliation)

PI: Ivan N. Mefford, Section on Clinical Pharmacology, ET, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Experimental Therapeutics Branch (Formerly: Clinical Neuroscience Branch)

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

PROJECT DISCONTINUED DUE TO PRINCIPAL INVESTIGATOR'S DEPARTURE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PERIOD COVERED
October 1, 1991, through September 30, 1992

ZOT MH 02486-04 ETB

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Action of Antidepressants

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: William Z. Potter	Chief	SCP, ETB, NIMH
Others: H. Manji	Visiting Associate	SCP, ETB, NIMH
G. Chen	Visiting Fellow	SCP, ETB, NIMH
A.M. Spiegel	Chief	MPB, NIDDK
D.L. Alkon	Chief	NSB, NINDS
D.L. Murphy	Chief	LCS, NIMH

COOPERATING UNITS (if any)

Laboratory of Clinical Science, NIMH; Laboratory of Biorganic Chemistry, NIDDK;
Laboratory of Clinical Studies, NIAAA

LAB/BRANCH

Clinical Neuroscience Branch* (transfer to Experimental Therapeutics Branch 10-1-91)

SECTION

Section on Clinical Pharmacology

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:

3.45

PROFESSIONAL:

3.0

OTHER:

0.45

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

The preclinical unit has focused on elucidating the molecular mechanism(s) of various antidepressant and antimanic treatments. Highlights of the last year include:

1. In vitro and ex vitro studies in rat brain have clearly demonstrated that protein kinase C (PKC) is a target for lithium's actions. Using [³H] PDBu as a quantitative autoradiographic radioligand for PKC, we have demonstrated that chronic (but not acute) lithium treatment produces a marked and significant reduction in [³H] PDBu binding in several hippocampal structures, most notably the CA1 region and subiculum. Immunolabeling with monoclonal antibodies has shown that this is largely due to an isozyme-specific reduction in membrane-associated PKC α .
2. In vivo microdialysis of cyclic AMP has shown that chronic lithium increases basal cAMP in rat cortex and hippocampus, while markedly attenuating the response to infused phorbol esters. Ex vivo studies have shown that chronic lithium treatment results in similar effects as phorbol ester treatment - a shift in the sensitivity of the guanine nucleotide/Gi heterotrimer dissociation curve to the left.
3. Chronic treatment of rats with different classes of antidepressants reduces the levels of G α and G α _{i,3} in hippocampus (as assessed by ELISA studies), while producing a complex pattern of effects on G α , mRNA, G α _{i,3} mRNA, G α , mRNA, and G α , mRNA levels, suggesting that the delayed therapeutic efficacy of these drugs may be due to transcriptional regulation of G protein α subunits.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02177-10 ET
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Behavioral Functions of Neuropeptides		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.N. Crawley, Chief, Unit of Behavioral Neuropharmacology, ETB, NIMH Others: R. Corwin, Guest Research ETB, NIMH J. Robinson, Staff Fellow ETB, NIMH P. Holmes, Staff Fellow ETB, NIMH G. Hodziewicz, ADAMHA Summer Teacher Program ETB, NIMH A. Jorn, NIH Normal Volunteer, Student ETB, NIMH R. Lawande, NIH Summer Student ETB, NIMH V. Koprivica, Student Volunteer ETB, NIMH		
COOPERATING UNITS (if any) 		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Unit on Behavioral Neuropharmacology, ETB, NIMH		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center;">5.0</div>	PROFESSIONAL: <div style="text-align: center;">3.1</div>	OTHER: <div style="text-align: center;">1.9</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A. <u>Galanin</u> produced a profile similar to scopolamine and MK-801 on the delayed non-matching to sample <u>memory</u> task. Galanin decreased choice accuracy on DNMTS when microinjected into the lateral ventricle, ventral hippocampus, and nucleus basalis magnocellularis of rats. Galanin was found to stimulate <u>feeding</u> when microinjected into the paraventricular nucleus of the hypothalamus and central nucleus of the amygdala. New galanin <u>antagonists</u>, C7, M40, and M32, blocked galanin-induced feeding when administered intraventricularly or into the paraventricular nucleus of the hypothalamus, at doses of antagonists equimolar to or lower than galanin doses.</p> <p>B. <u>Push-pull perfusion</u> was found to have considerably higher recoveries than <u>microdialysis</u>, and allowed detection by standard radioimmunoassay of both <u>cholecystokinin</u> and <u>galanin</u>. Split samples from the nucleus accumbens allowed measurement of dopamine by HPLC and CCK by RIA in the same sampling period, in a feeding paradigm in rats. A CCK-B antagonist increased dopamine concentrations in microdialysate from the <u>nucleus accumbens</u> of anesthetized rats. 50 mM potassium stimulation increased galanin concentrations in push-pull perfusate from the <u>ventral hippocampus</u> of anesthetized rats.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="text-align: right; padding-top: 10px;">Z01 MH 02178-10 ET</div>
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacology of Anxiety		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.N. Crawley, Chief, Unit of Behavioral Neuropharmacology, ETB, NIMH Others: C. Mathis, Fogarty Fellow ETB, NIMH J. Buckholtz, Student Volunteer ETB, NIMH		
COOPERATING UNITS (if any) Section on Molecular Pharmacology, Clinical Neuroscience Branch		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Unit on Behavioral Neuropharmacology, ETB, NIMH		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: <div style="text-align: center; padding-top: 5px;">1.0</div>	PROFESSIONAL: <div style="text-align: center; padding-top: 5px;">0.75</div>	OTHER: <div style="text-align: center; padding-top: 5px;">0.25</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <div style="padding: 10px;"> <p>Genetic analysis of anxiety-related behaviors has begun for recombinant inbred strains of the C57Bl/6J x A/J cross. A screen of mouse strains showed the biggest behavioral differences between the C57Bl/6J and the A/J from Jackson Labs. This cross is also a good choice because 30 RI strains are maintained at Jackson from this cross. Baseline light-dark transitions, increase in transitions after diazepam, open field ambulation, were found to be high in the C57Bl/6J and low in the A/J. The A/J were more sensitive to pentylenetetrazol and to B-CCM on induction of seizures. Behavioral testing on these parameters has been completed for 11 RI strains, 9 are partially tested, and the remaining strains are now arriving, to create a data base sufficient for statistical analysis of number of genes which may be mediating anxiety-related behaviors in mice.</p> </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02179-10 ET
PERIOD COVERED October 1, 1991 to September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Animal Models of Neuropsychiatric Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) PI: J.N. Crawley, Chief, Unit of Behavioral Neuropharmacology, ETB, NIMH Others: A. De Bartolomeis, Fogarty Visiting Fellow ETB, NIMH V. Koprivica, Student Volunteer ETB, NIMH R. Lawande, NIH Summer Student		
COOPERATING UNITS (if any) 		
LAB/BRANCH Experimental Therapeutics Branch		
SECTION Unit on Behavioral Neuropharmacology, ETB, NIMH		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20892		
TOTAL MAN-YEARS: 1.75	PROFESSIONAL: 1.25	OTHER: 0.50
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p> <u>In situ</u> hybridization analysis of mRNAs for <u>tyrosine hydroxylase</u>, <u>cholecystokinin</u>, <u>enkephalin</u>, the <u>D-1</u> and <u>D-2 dopamine receptor subtypes</u>, and the <u>dopamine transporter</u>, in brains from rats treated neonatally with cocaine, are almost completed. No difference between cocaine treatment and dietary controls has been seen in mRNA concentrations in the ventral tegmentum, substantia nigra, caudate nucleus, or nucleus accumbens, for TH, CCK, ENK, D-1, or D-2 mRNAs. Dr. de Bartolomeis has recently developed a new oligonucleotide probe for the dopamine transporter, which he has characterized for specificity, and is now quantitating in the <u>prenatal cocaine</u> experiment. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02231-08 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological-Behavioral Relations in Early Adolescence

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E.D. Nottelmann	Research Psychologist	CADRB
OTHERS:	G.I. Germain	Research Psychologist	LDP NIMH
	L.D. Dorn	Quest Researcher	LDP NIMH
	E.J. Susman	Quest Researcher	LDP NIMH
	G.B. Cutler, Jr.	Senior Investigator	DEB NICHD
	G.P. Chrousos	Senior Investigator	DEB NICHD

COOPERATING UNITS (if any)

Developmental Endocrinology Branch, NICHD
Child and Adolescence Disorders Research Branch

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Principle Investigator transferred from Laboratory of Developmental Psychology.
Project terminated. This is a final report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02361-06 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Multimethod Assessments of Children's Psychosocial Development

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P.E. Martinez Senior Staff Fellow LDP NIMH

Co-PI: L. Tarullo Staff Fellow LDP NIMH

Others: M. Radke-Yarrow Chief LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.90

PROFESSIONAL:

.60

OTHER:

.30

CHECK APPROPRIATE BOXES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Assessment of the psychiatric status of children poses difficult problems for research and clinical evaluation. Different data sources often yield different evaluations. In the longitudinal study of children of affectively ill and well parents, we have utilized a multi-source and multi-method approach in assessing the children. Of particular interest are comparisons of psychiatric interview assessments based on information from child, mother, and father.

Project Description:

Assessment of children's psychiatric status poses difficult problems for research and clinical evaluation. Different data sources often yield different evaluations. In the longitudinal study of children of affectively ill and well parents, we have utilized a multi-source and multimethod approach in assessing the children. DICA interviews were used. Two children in each family (one between 8 and 10 years, the other between 11 and 15 years) were studied. All families were participants in a longitudinal study. Analyses are directed to the following questions: 1) How do parents compare; 2) How does each parent compare with child?; 3) How are comparisons influenced by gender, age, psychiatric status of informants, kind of problem being reported?

Methods and Findings:

Psychiatric structured interviews were used with the children (DICA) and the parents (DICA-P). Two children in each family (one between 8 and 10 years, the other between 11 and 15 years) were studied. All the families were participants in a longitudinal study.

Significance to Biomedical Research:

Early and valid assessment of present problems and precursors of later problems in each children is important for understanding the course of development, helping to identify etiological factors, and aiding in prevention.

Proposed Course:

Analyses are underway.

Publications:

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02365-06 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Psychobiological Effects of Sexual Abuse

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator: F. W. Putnam

Other Investigators:

P. Trickett Guest Researcher

LDP NIMH

G. Chrousos NICHD

COOPERATING UNITS (if any)

Chesapeake Institute, Wheaton, MD; NICHD

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.75

PROFESSIONAL:

1.00

OTHER:

.75

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is comprised of a series of studies investigating the psychological and biological effects of sexual abuse in female children. Subjects are sexually abused girls, aged 6-15 years at time of entry into the study. Control subjects are matched on age, race, socioeconomic status and family situation (one or two parent household). The project uses a multi-method approach to data acquisition. New findings include further conformation of significantly higher rates of major psychiatric disorders in abused children, significantly higher levels of dissociation, which is strongly correlated with psychopathology, and elevated morning serum cortisol levels. Hypnotizability does not differ significantly between the two groups indicating that autohypnosis is not the mechanism responsible for the increased dissociative behavior in the abused girl

Project Description

Objective: Childhood sexual abuse has recently been recognized as a national public health epidemic that makes a significant contribution to long-term mental health problems. Child sexual abuse is now implicated in the development of several psychiatric disorders including: Borderline personality disorder, eating disorders, somatoform disorders, multiple personality disorder and substance abuse disorder in women. Even non-clinical samples of adults sexually abused as children demonstrate high rates of depression, suicidality, substance abuse, violence toward others, somatic complaints, and sexual dysfunction. The scanty data available on the immediate effects in childhood find high rates of depression, running away and antisocial behavior, learning difficulties, attention deficit disorder, suicidal and self-destructive behavior, aggression and inappropriate sexual behaviors.

This project is the first study to prospectively follow the psychological and physical development of sexually abused children. Three sets of hypotheses are being tested: 1) that specific developmental milestones (e.g. puberty) exacerbate the symptomatology of sexually abused children compared to matched controls; 2) that certain behaviors common in abused children (i.e. aggression and hypersexuality) may result from biological alterations in abused children; and 3) that dissociative behavior, a psychophysiological response to trauma, is higher in abused children and strongly associated with pathological behaviors.

Methods Employed: The study uses a cross-sequential design that permits the data to be analyzed both cross-sectionally and longitudinally. In addition, longitudinal analyses are conducted by time of evaluation, developmental stage and chronological age. Psychological development is assessed by a variety of standardized measures of competence and coping and of psychopathology. Pathology measures assess both dimensional constructs and DSM-III-R diagnoses. Physical measures include Tanner staging, height and weight and serial hormonal levels. Dissociation is measured by standardized hypnosis scales, an blindly-rated observational measure and the Child Dissociative Checklist (CDC) developed by LDP, NIMH, DIRP.

New Findings:

As of this report, approximately 170 children have been evaluated for Time 1 of the study and about 120 children for Time 2 and almost 70 children have been seen for Time 3. Preliminary analyses of the data demonstrate robust differences between the abuse and control group on a wide range of measures. There are significant differences in number and types of DSM-III-R diagnoses made by the Diagnostic Interview for Children and Adolescents (DICA), a structured interview. Abused girls had significantly higher rates of Attention Deficit Hyperactivity Disorder (ADHD), Conduct Disorder, Major Depressive Disorder and Dysthymic Disorder compared to matched controls. There were significant differences between the groups on Child Dissociative Checklist scores (Mann Whitney $Z=3.8$, $p = .0001$). Dissociation scores are significantly correlated with many measures of pathology in both abuse and control groups and are an important dimension underlying disturbed behavior. Hypnotizability, as assessed by blindly rated standardized measures, does not differ between the two groups. This eliminates autohypnosis as the mechanism for increased dissociation in the abused group.

Sexually abused girls had significantly higher morning cortisol levels. There were also significant differences between the abuse and control groups for every item of the Teacher Child Rating Scale (TCRS) and on 6 out of 8 subscales on the Child Behavioral Checklist (CBCL). Blind ratings of videotapes of social behaviors in the presence of a strange male (psychologist, blind to group, administering paper and pencil tests to child) demonstrate extraordinary differences in the behaviors

of abused children compared to controls. Abused children were significantly more sexualized and personally revealing in their interactions with a stranger than control children.

Significance to Mental Health Research

This study is the first longitudinal research project to prospectively investigate specific biological mechanisms for behavioral and psychiatric problems frequently observed in sexually abused children. A number of potential areas of intervention have been identified that can be tested in future studies which include a treatment component.

Proposed Course

The current study is supported by both an extramural grant and intramural NIMH funding. Data collection is scheduled to end July 1, 1993. If additional funding can be obtained, a five year follow-up will be conducted to extend the longitudinal data on outcome of sexual abuse.

Publications

Putnam, FW: Dissociative disorders in children and adolescents: A developmental perspective. **Psychiatric Clinics of North America** 14:519-521, 1991

Cole, P, Putnam, FW: The effect of incest on self and social functioning: A developmental psychopathology perspective. **Journal of Consulting and Clinical Psychology**, 60:1-9, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02366-06 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Psychophysiology of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator: Frank W. Putnam, LDP, NIMH

Other Investigators:

H. Weingartner Chief, Cognitive Neuroscience Section, NIAAA;

Pam Cole LDP, NIMH;

John Bartko Biometric and Statistics Branch, NIMH DIRP.

COOPERATING UNITS (if any)

Cooperating Units: NIAAA, Biometric and Statistics Branch, NIMH DIRP.

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.85

PROFESSIONAL:

.60

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project seeks to characterize and quantify cognitive and physiological differences that occur between alter personality states of patients with multiple personality disorder. Alter personalities are studied using a variety of methodologies and technologies. Current efforts are directed at developing a computerized coding system using image processing software for analysis of facial emotional displays.

Project Description

Objectives: This project investigates the differential psychological and physiological correlates found across the alter personality states of patients with multiple personality disorder.

Methods Employed: A range of methodologies and technologies have been used to investigate the cognitive and physiological differences reported by clinicians working with these patients. These methods include: visual evoked potentials, auditory evoked potentials, galvanic skin response, xenon-inhalation cerebral blood flow, continuous telemetry EEG, indwelling venous catheter sampling, neuropsychological memory testing, and computer image analysis of facial displays. Changes between alter personality states are compared to simulating control subjects and to controls in altered states of consciousness induced by hypnosis or deep relaxation.

New Findings:

Using image processing software (Image V. 1.43) developed by the Research Services Branch, NIMH, photographs of the facial displays of different alter personalities are being analyzed. A number of strategies are being investigated for the quantification and statistical analysis of facial display. This computerized approach appears to be very sensitive to differences in facial display

Significance to Mental Health Research

The development of reliable and valid computerized quantification of facial emotional displays could become a very powerful tool for extracting data on affect from videotape records. At present, data of this nature are collected through observer ratings that are laborious and time-consuming. For example, it takes approximately one hour to code one minute of videotape using the current Eckman rating system. Observer rating systems for affect are subjective in nature and require extensive attention to interrater reliability to maintain validity. Computerization of this process would allow standardized measurements to be rapidly made on a series of frames, thereby permitting intensive sampling with commensurate increases in statistical power.

Proposed Course:

The computerized facial emotional coding project is in a developmental stage at present. A variety of techniques are being tried and evaluated in consultation with Dr. John Bartko, statistical consultant. Materials being examined include still photographs of alter personality states from MPD subjects, still photographs of bipolar patients in depressed, manic and euthymic states and photographs published as part of the Eckman facial coding system. If analyses of these materials proved successful, then videotape material will be tested. The goal is to produce a proof of concept paper.

Publications

Demitrack MA, Putnam FW, Pigott TA, Altemus M, Krahn DD, Gold PW. Relation of dissociativity to levels of cerebrospinal fluid monoamine metabolites and β -endorphin in patients with eating disorders. *Biological Psychiatry* (in press)

Putnam, FW: Recent research on multiple personality disorder. *Psychiatric Clinics of North America* 14:489-502, 1991.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02367-06 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Clinical Phenomenology of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Principal Investigator: Frank W. Putnam

Others Investigators:

R. Loewenstein Assoc Clinical Professor Psychiatry, Univ of Maryland;

N. Hornstein Asst Professor Psychiatry, UCLA, Los Angeles;

G. Peterson Assoc Professor Psychiatry, Univ of NC, Chapel Hill, NC;

P. Siraganian Psychiatry Resident, Univ of Maryland.

COOPERATING UNITS (if any)

Cooperating Units: Sheppard Pratt Hospital, Baltimore, MD; Dept of Psychiatry and Behavioral Sciences, UCLA; Dept of Psychiatry, UNC; Dept of Psychiatry, Univ of Maryland

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20982

TOTAL STAFF YEARS:

.65

PROFESSIONAL:

.40

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project seeks to document the clinical phenomenology of dissociative disorders in children, adolescents and adults. Findings from a recent interview study of children and adolescents with dissociative disorders provide the first systematic documentation of these diagnoses in youth. Important results include the high rates of dissociative, affective and posttraumatic symptoms that parallel clinical presentations in adults.

Project Description

Objectives: Recent research using the Dissociative Experiences Scale (a measure developed in the IRP) found that approximately 5% of inpatients admitted to psychiatric units suffer from multiple personality disorder (MPD). MPD is highly associated with a history of severe childhood abuse and is increasingly being reported in children and adolescents. The purpose of this project is to characterize the disorder in children, adolescents and adults, to determine the range of clinical presentations and the responses of patients to standard treatments.

Methods Employed: This project has relied on standardized questionnaires developed by the DIRP and other organizations to collect information on cases meeting DSM-III-R/NIMH diagnostic criteria. See Annual Reports 1988, 1989 for description of methods.

In collaboration with LDP, Dr. Patricia Siraganian, Dept of Psychiatry, University of Maryland, is conducting a structured interview study to compare the clinical phenomenology of Dissociative Disorder Not Otherwise Specified (DDNOS) and multiple personality disorder (MPD) patients. Instruments used in the study include: Dissociative Experiences Scale (DES), Dissociative Disorders Interview Schedule (DDIS), Childhood Antecedents Questionnaire, PTSD structured interview, Hopkins symptom checklist 90R, Beck Depression Inventory, Spielberger State-Trait Anxiety Scale and a clinician questionnaire for MPD developed in LDP. Interviews are being conducted by Dr. Sariganian at the University of Maryland College Park campus under the auspices of the Dept of Psychiatry. Dr. Putnam, LDP, NIMH will assist in the analysis and presentation of the data.

New Findings:

A study of 64 children and adolescents with working diagnoses of multiple personality disorder (MPD) or Dissociative Disorder Not Otherwise Specified (DDNOS) interviewed at two separate sites demonstrated strong agreement for these diagnostic constructs in children. Compared with DDNOS children, MPD cases were found to be significantly more symptomatic for amnesia symptoms (Mann Whitney $Z = 4.44$, $p = .0001$), hallucinations ($Z = 3.152$, $p = .001$) and identity disturbances ($Z = 5.07$, $p = .0001$). Child Dissociative Checklist (CDC) scores were also significantly different between the two diagnostic groups (MPD mean = 25.16, DDNOS = 16.63, $p = .0001$). The CDC is an observer report scale measuring dissociative behaviors in children developed in the Laboratory of Developmental Psychology, DIRP. Clinically these children and adolescents demonstrated an array of affective, posttraumatic, hallucinatory and process symptoms that were strikingly similar to adult dissociative disorder clinical presentations.

Significance to Mental Health Research

This is the first large sample of children and adolescence systematically evaluated for dissociative symptoms and for general psychiatric symptoms. This study validates the use of adult DSM-III-R dissociative disorder diagnoses in children and adolescents. In addition, this is the first large scale study of the clinical phenomenology of dissociative disorders in children and adolescents.

The study by Dr. Saraganian is the first systematic structured interview study of the differences and similarities of MPD and DDNOS cases.

Proposed Course

A second sample of children and adolescents with dissociative disorders is being collected by questionnaire by Dr. Peterson, Dept of Psychiatry, University of North Carolina. Questionnaires

based on the above interview sample and developed in LDP have been distributed by Dr. Peterson to clinicians around the country treating children or adolescents with dissociative symptoms. Data from this study will be sent to LDP for analysis and comparison with the above interview sample data. The two samples will be compared to cross-validate the clinical phenomenology and diagnostic validity of these two dissociative disorders in children and adolescents.

Publications

Quimby, LG, and Putnam, FW: Dissociative symptoms and aggression in a state mental hospital. *Dissociation*, 4:21-24, 1991.

Hornstein NL and Putnam FW. Clinical phenomenology of child and adolescent dissociative disorders. *Journal of the American Academy of Child and Adolescent Psychiatry*. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02368-06 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Dissociative Experiences Scale (DES)

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: F. Putnam Staff psychiatrist LDP NIMH

OTHERS: E. Carlson Asst. Professor Psychology Beloit College
Beloit, WI

COOPERATING UNITS (if any)

Beloit College

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Data incorporated under project Z01 MH 02367-06. This is a final report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02369-06 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mutual Mother-Child Influences in Families With and Without Affective Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Kochanska Associate Professor State Univ. of Iowa

COOPERATING UNITS (if any)

State University of Iowa

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Principle Investigator transferred from Laboratory of Developmental Psychology, NIMH. Project terminated. This is a final report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02370-06LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Caregiving Patterns in Stressed Families

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Radke-Yarrow

Chief

LDP NIMH

OTHERS: D. Hay

Research Psychologist

U. of London

T. Cox

Psychiatrist

U. of Liverpool

COOPERATING UNITS (if any)

University of London

University of Liverpool

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Data from these analyses are being incorporated in Project Number Z01 MH 02491-03.
This is a final report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02372-06 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychiatric Status of Children of Depressed Parents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Radke-Yarrow Chief LDP NIMH

Others: E. Nottelmann Statistician LDP NIMH
M. Fox Psychiatrist Private Practice
Palo Alto, CA

P. Martinez Senior Staff Fellow LDP NIMH
B. Belmont Social Science Analyst LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.40

PROFESSIONAL:

.60

OTHER:

1.80

CHECK APPROPRIATE BOXES:

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project terminated

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 MH 02381-06 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Functioning of Depressed Mothers In and Between Episodes

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	M. Radke-Yarrow	Chief	LDP NIMH
CO-PI:	P. Martinez	Senior Staff Fellow	LDP NIMH
	E. DeMulder	Staff Fellow	LDP NIMH
	L. Tarullo	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.10

PROFESSIONAL:

.10

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

A diagnosis of depression includes much heterogeneity in etiology, severity of illness, symptom patterns, timing of episodes, treatment, personality, and behavioral functioning. From data available in the NIMH longitudinal study of families of affectively ill and well parents, it is possible to examine differences within maternal depression and relate them to child functioning: (1) Do certain symptom patterns have greater effect on children than others? (2) Are there residues of illness present between episodes? (3) How do mothers' personality characteristics or disorders have an impact on associations with children's problems? Recency of mother's episodes does not have a strong over-all effect on mother-child interactions. With older daughters, recency is associated with more negative interaction.

Project Description:

Depression defines a group of mothers who meet certain diagnostic criteria (RDC or DSM-III). This definition leaves much heterogeneity, however, in etiology, severity of illness, symptom patterns, timing of episodes, treatment, personality, and behavioral functioning. In order to understand influences of maternal affective illness on children's problems, differences within the diagnosis of depression need to be taken into account.

From data available in the NIMH longitudinal study of families of affectively ill and well parents, it is possible to examine differences within maternal depression and relate them to child functioning: (1) Do certain symptom patterns have greater effect on children than others? (2) Are there residues of illness in patterns of parenting between episodes? (3) How do mothers' personality characteristics or disorders, in addition to the diagnosis of depression, impact on children?

Methods and Findings:

The parents and two siblings in each family participated in the longitudinal study. Data for these analyses were obtained when the younger siblings were between 8 and 10 years and the older siblings between 12 and 16 years. Parents were given the SCID interview and PDE (Personality Disorders Examination). Children were diagnosed using the DICA and DICA-P. Behavioral observations of mother-child interaction were also made.

Mothers within episodes in the past month and mothers with less recent episodes were compared in their interactions with each sibling. Recency of episode had no effect on the interactions of mothers and sons. With the younger sibling, children of mothers not in episode were significantly less comfortable in their interactions with mother than children of well mothers. With the older daughters, recency of mother's episode was associated with more critical and irritable mother-child interaction.

Significance to Biomedical Research:

Evaluation of the course of children's problems in relation to parental illness and stressful family circumstances should provide information on the interaction of parental diagnosis, child's developmental stage, and family experiences in influencing the child's functioning. Also, questions concerning the state/trait dependence of maternal behavior are of crucial importance to an understanding of the links between maternal depression and offspring adjustment.

Proposed Course:

Analyses have been completed and have been incorporated in a manuscript submitted for publication (MH 02488). Analyses of other aspects of maternal depression are ongoing.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02408-05 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Competency in Children at Risk for Psychiatric Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K. Free	Staff Fellow	LDP NIMH
OTHERS:	W. Habelow	Research Psychologist	LDP NIMH
	E. Nottelmann	Statistician	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The investigators on this project are no longer in the IRP-NIMH staff. The project is closed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02409-05 LDP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Differential Development of Siblings in Shared and Nonshared Family Environments		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> E. Nottelmann Research Psychologist CADRB </div>		
COOPERATING UNITS (if any)		
LAB/BRANCH Child and Adolescence Disorders Research Branch		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p>Children in the same family often differ significantly in psychological characteristics and behavioral outcomes. They also show resemblances. However, the processes by which family environments are linked to sibling differences and similarities are only partly understood. Such an understanding is critical to developmental theory and to theories of constitution-environment interactions. The purpose of this study is to investigate the <u>shared and nonshared family experiences of siblings</u> in relation to their characteristics and development. The research objectives are: to investigate <u>child attributes</u> (temperament, gender, age, etc.) that relate to <u>differential treatment by parents</u> and to investigate links between siblings' shared and nonshared family experience and the <u>developmental course of each sibling</u>.</p>		

Project Description:

Children in the same family often differ significantly in psychological characteristics and behavioral outcomes. They also show resemblances. However, the processes by which family environments are linked to sibling differences and similarities are only partly understood. Such an understanding is critical to developmental theory and to theories of constitution-environment interactions. The purpose of this study is to investigate the shared and nonshared family experiences of siblings in relation to their characteristics and development. The research objectives are: to investigate child attributes (temperament, gender, age, etc.) that relate to differential treatment by parents and to investigate links between siblings' shared and nonshared family experience and the developmental course of each sibling.

Methods and Findings:

The participants were 40 families (mother, father, and 5-to 6-year old and 8-to 11-year old siblings) enrolled in the longitudinal study (MH-02155) of offspring of affectively ill and well parents. Detailed observations were made from a videotaped record of their visit to the laboratory apartment, focusing on unstructured interaction (the four family members were free to use the time in any way they chose). Each individual's behavior was examined in relation to every other family member.

Twenty families with a relatively low chronic stress level and no history of affective illness and 20 families with a relatively high chronic stress level and maternal affective illness were studied. The sibling constellation was balanced by age and gender, (10 both male, 10 both female, 10 older boy-younger girl, 10 older girl-younger boy).

Proposed Course:

No staff time was devoted to this project during the year. Analyses will proceed this coming year.

Significance of Biomedical Research:

The family environment is not the same set of experiences for each child, but little evidence exists regarding the factors or conditions that determine differential treatment of siblings and the consequences of these differences for children's development. This is particularly important in dysfunctional families in which certain children are nonpreferred or victimized.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02410-05 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Observational Assessment of Parent and Child from Toddlerhood to Late Childhood

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Nottelmann	Research Psychologist	CADRB
CO-PI:	M. Radke-Yarrow	Chief	LDP NIMH
OTHERS:	L. Tarullo	Staff Fellow	LDP NIMH
	B. DeMulder	Staff Fellow	LDP NIMH

COOPERATING UNITS (if any)

CADRB

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.05

PROFESSIONAL:

.35

OTHER:

.70

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The objective is to investigate the mother-child relationship from three perspectives: to identify the mutual (mother and child) contributions to the relationship, to develop alternative frameworks for conceptualizing the relationship, and to examine the developmental, stabilities and changes in the relationship over a 8-year period. Comparisons of families with and without parental psychopathology are a second objective. Twenty families with affectively ill parents and 20 families with well parents are studied. There are two children (average age 2 1/2 and 6 years) in each family. Families are assessed twice, three years apart. Behavioral observations over systematically sampled situations and at multiple times are obtained. Coding is at a detailed level and at level involving clinical interpretation.

Project Description:

The objective is to investigate the mother-child relationship from three perspectives: to identify the mutual (mother and child) contributions to the relationship, to develop alternative frameworks for conceptualizing the relationship, and to examine the developmental, stabilities and changes in the relationship over a 8-year period. Comparisons of families with and without parental psychopathology are a second objective.

Methods and Findings:

Twenty families with affectively ill parents and 20 families with well parents are studied. There are two children in each family, average ages 2 1/2 and 6 years at the beginning of the study. The age and gender configurations are identical in the ill and well families. Behavioral observations over systematically sampled situations at multiple times are obtained. Assessments are made twice, three years apart. Coding is done at a detailed level and at a level involving clinical judgement. Coders are not informed as to the diagnostic status of the parents.

Significance to Biomedical Research:

The contribution of the mother-child relationship to the well or problem status of the child is well documented. The security of this relationship (as in attachment) has been a primary focus. We assume that a more differentiated conceptualization and measurement of the multiple relationships in mother's and child's engagement with each other would add significantly to the predictive power of maternal variables in child development.

Proposed Course:

The final cases are being coded. The first analytic focus is on the mutual influences of parent and child on each other.

Publications:

Germin G.I., Nottelmann, E.D., and Radke-Yarrow, M. (1992). Evaluative communications between affectively ill and well mothers and their children. Journal of Abnormal Child Psychology, 20(2), 189-212.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02411-05 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Sleep Disturbances in Young Children of Mothers with an Affective Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: E. Nottelmann Research Psychologist CADRB

OTHERS: S. Stoleru Chef du Clinique Institute National
Asst. de la Recherche
Medicale, Le
Kremlin-Bicetre,
France

COOPERATING UNITS (if any)

Institut National de la Recherche Medicale, Le Kremlin-Bicetre, France
Child and Adolescence Disorders Research Branch

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Principal Investigator transferred from Laboratory of Developmental Psychology.
Project terminated. This is a final report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02442-04 LDP												
PERIOD COVERED October 1, 1991 through September 30, 1992														
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Suicidal Thinking in the Children of Depressed and Well Parents														
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: K. Free</td> <td style="width: 33%;">Staff Fellow</td> <td style="width: 33%;">LDP NIMH</td> </tr> <tr> <td>OTHERS: G. Brown</td> <td>Clinical Director</td> <td>NIAAA</td> </tr> <tr> <td>R. Rawlings</td> <td>Statistician</td> <td>LCS NIAAA</td> </tr> <tr> <td>M. Radke-Yarrow</td> <td>Chief</td> <td>LDP NIMH</td> </tr> </table>			PI: K. Free	Staff Fellow	LDP NIMH	OTHERS: G. Brown	Clinical Director	NIAAA	R. Rawlings	Statistician	LCS NIAAA	M. Radke-Yarrow	Chief	LDP NIMH
PI: K. Free	Staff Fellow	LDP NIMH												
OTHERS: G. Brown	Clinical Director	NIAAA												
R. Rawlings	Statistician	LCS NIAAA												
M. Radke-Yarrow	Chief	LDP NIMH												
COOPERATING UNITS (if any) Laboratory of Clinical Studies NIAAA														
LAB/BRANCH Laboratory of Developmental Psychology														
SECTION														
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892														
TOTAL STAFF YEARS: .05	PROFESSIONAL: .05	OTHER:												
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews														
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The aims of this study are: 1) to investigate the occurrence and nature of <u>children's suicidal thoughts and gestures</u>; 2) to study the related factors in maternal diagnostic characteristics, and 3) to compare the clinical, developmental, demographic, age, and gender characteristics of suicidal and non-suicidal children. In a longitudinal study, 115 biological sibling pairs from three maternal diagnostic categories, Controls, Unipolars, Bipolars, were seen at three times across six years. Assessments of mothers were made from psychiatric interviews (SADS-L, RDC Criteria); assessments of children's were made using psychiatric interviews (CAS at Times I and II and DICA at Time 3). Among older siblings at Time III, ratings were significantly different overall ($p < .03$; between control vs. bipolar groups, ratings of bipolar offspring were significantly higher ($p < .05$). Suicidal behavior in offspring was found to relate to <u>maternal psychopathology</u> and child's age, but not gender. </p> <p>This is a final report.</p>														

Project Description:

The primary question proposed in this study was: Do offspring of mothers without history of psychiatric diagnoses vs. offspring of those with history of major affective disorder, unipolar or bipolar, develop differences in suicidal ideation and/or attempts?

Methods and Findings:

In a longitudinal study, 115 biological sibling pairs from three maternal diagnostic categories--Controls, Unipolar, Bipolar--were seen at three times across six years (two three year intervals). Assessments of the mothers were made from standard, structured psychiatric interviews (SADS-L), blind administered and blind-rated, using the Research Diagnostic Criteria (RDC) to establish the presence of major affective disorder. Assessments of suicidality in the children were made from standard, instrument item-based, psychiatric interviews, blind-administered and blind-scored (Child Assessment Schedule (CAS) at Times I and II, the Diagnostic Interview for Children/Adolescents (Revised) and for Parents (Revised) (DICA C/A and DICA-P at Time III). The reports of behavior relevant to suicide were classified as: Type I--suicidal ideation (general thinking about killing oneself); Type II--suicidal ideation (suicide plan/minor self-destructive behavior); Type III--suicidal attempts (significantly self-destructive behaviors inflicting bodily harm accompanied by suicidal ideation; and Type IV--suicide completion.

Statistical analyses included non-parametric Kruskal-Wallis one-way ANOVAS (for categorical group data) and non-parametric Friedman two-way ANOVAs (for within child, across time data). Among older siblings at Time III, ratings were significantly different overall ($p = .03$); between control vs. bipolar groups, ratings of bipolar offspring were significantly higher ($p < .05$). Suicidal behavior in offspring was found to relate to maternal psychopathology and child's age, but not gender.

There were several observations of the mother/sibling pairs that were not reflected in statistical analyses. Mothers who had at least one child to report suicidal ideation and/or behavior were 28% of the controls, 53% of the unipolars, and 58% of the bipolars; those who had both siblings to report these clinical findings were 4%, 20% and 15% respectively; those who had a child to report these same clinical findings at more than one time point, representing more than one clinical episode were 4%, 24% and 15% respectively.

According to these data it would appear that all of the suicide attempters at Time III had a mother with a Major Affective Disorder; three of the six attempters had earlier reported thinking about suicide, and five had reported symptoms which met criteria for a diagnosis at either Time I or II. The two male attempters were characterized (mother's report) as "aggressive, uncontrolled and unpredictable" (confirmed by interviewer's clinical note); the four female attempters were characteristically "moody and depressed" (mother's report). All four of the females were described (mother's report) as "artistic;" all wrote poetry and had other talents; two of the attempters had published poems with depressive themes. There was a wide range in IQ scores for the children who attempted suicide (from Below Average to Superior); therefore, trends in the cognitive functioning of attempters could not be identified.

Proposed Course:

This is a final report.

Significance to Biomedical Research:

Tragically, child and adolescent suicide is the final outcome of serious, often unrecognized and untreated, psychiatric disorder. It is the aim of this study to contribute information relevant to crucial

questions regarding the prevalence of suicidal thinking in childhood, to describe the patterns of premorbid adjustment, and to identify the clinical precursors of suicidal children.

Publications:

Manuscript submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02443-04 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Potential Impact of Psychiatric Treatment Upon Mother-Child Communication

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	K Free	Staff Fellow	LDP NIMH
OTHERS:	W Habelow	Research Psychologist	LDP NIMH
	P Cole	Senior Staff Fellow	LDP NIMH
	C Zahn-Waxler	Chief, Section on	LDP NIMH
		Child Behavior Disorders	
	M Radke-Yarrow	Chief	LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Affective Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.05

PROFESSIONAL:

.05

OTHER:

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Affective language between depressed mothers and their children is examined to determine whether differences in mothers' psychiatric treatment corresponds to differences in their ability to talk with their children about feelings. This involves assessing 1) mother's accuracy in interpreting emotional expression to her child, 2) mother's ability to correct her child's inaccurate interpretation of facial expressions, 3) mother's ability to qualify and elaborate discussion in emotion terms, and 4) the child's accuracy in interpreting emotional expression to mother. Verbal communication about emotion was assessed for accuracy in 84 mother/child pairs from three maternal categories, Control Depressed with psychiatric treatment and Depressed without treatment. Mothers were diagnosed using the SADS-L and scored according to RDC. History of psychiatric treatment was obtained during the diagnostic interview. Mother/child verbal communications about emotions were assessed using eight pictures of infants expressing emotions through facial expression. The mother/child discussion of these pictures was scored for accuracy. A comparison by ANOVA established that depressed mothers who have had psychotherapy were more accurate in communicating with their children about emotion than were depressed mothers with no treatment ($p < .03$); depressed mothers with treatment were most accurate in communicating about negative emotions specifically, and differed from the depressed mothers with no treatment ($p < .01$); there were no significant differences between control mothers and depressed mothers with a history of psychiatric treatment in accuracy of affective communication.

This is a final report.

Project Description:

Affective language between depressed mothers and their children was examined to determine whether differences in mothers' psychiatric treatment corresponds to differences in their ability to talk with their children about feelings. Mother/child communication about affect involved an assessment of 1) mother's accuracy in interpreting emotional expression to her child, 2) the child's accuracy in interpreting emotional expression to mother, and 3) mother's ability to correct her child's inaccurate interpretation of facial expressions.

Methods and Findings:

Verbal communication about emotion was assessed for accuracy in 84 mother/child pairs from three maternal diagnostic categories, Control, Depressed with psychiatric treatment, and Depressed without treatment. Mothers were diagnosed using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) and scored according to Research Diagnostic Criteria (RDC). Mothers who met criteria for a Major Affective Disorder were also rated for the severity of their illness (GAS scores from the SADS-L). The mothers' history of psychiatric treatment was obtained during the diagnostic interview.

Mother/child verbal communications about emotions were assessed using eight photographs (8"x 10") of infants expressing emotions through facial expression. The mother/child discussion of these pictures was scored for accuracy. A comparison by ANOVA established that depressed mothers who have had psychotherapy were more accurate in communicating with their children about emotion than were depressed mothers with no treatment ($p < .03$); depressed mothers with treatment were most accurate in communicating about negative emotions specifically, and differed from the depressed mothers with no treatment ($p < .01$); there were no significant differences between control mothers and depressed mothers with a history of psychiatric treatment in accuracy of affective communication.

An additional analyses (Chi-square) was performed which compared the severity of maternal depression in the two experimental groups (with and without treatment); the mothers from the treatment group were more severely ill than the mothers who had no treatment ($p < .0001$). Thus, mothers who have a severe depression were not impaired in their ability to talk accurately with their children about emotion.

Proposed Course:

This is a final report.

Significance to Biomedical Research and to the Program of the Institute:

This study brings together two relatively unexplored areas of human research on maternal affective disorder, i.e., maternal psychiatric treatment and mother/child communication about feelings. Findings contribute to a preliminary understanding of what mothers say to their children about emotion when they have had their own experience with depression; further, differences between those depressed mothers who have had psychotherapy vs. those who have not are presented. From a preventative perspective, this study furthers the exploration of how depressed mothers who have had psychotherapy assist their children in identifying emotions; such communication between mother and child may ultimately better equip the child to cope with an emotionally-laden child-rearing environment.

Publications:

Manuscript submitted to the Journal of the American Academy of Child and Adolescent Psychiatry, July, 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02444-04 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Temperament and Socialization in the Development of Guilt and Conscience

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Kochanska Associate Professor State U. of Iowa

COOPERATING UNITS (if any)

State University of Iowa

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Principal Investigator transferred from Laboratory of Developmental Psychology, NIMH. Project terminated. This is a final report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02446-04 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Postpartum Depression and the Development of Mother-Child Relations

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	E. Nottlemann	Research Psychologist	CADRB
OTHERS:	G. Chrousos	Senior Investigator	DEB NICHD
	D. Rabin	Quest Researcher	DEB NICHD
	P. Gold	Chief	CNE NIMH
	M. Kling	Medical Officer	CNE NIMH
	D. Rubinow	Clinical Director	NIMH

COOPERATING UNITS (if any)

Developmental Endocrinology Branch, NICHD
Biological psychiatry, NIMH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Principal Investigator transferred from Laboratory of Developmental Psychology.
Project terminated. This is a final report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02447-04 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Early Development of Prosocial and Antisocial Behavior

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Zahn-Waxler

Chief, Section on
Child Behavior Disorders

LDP NIMH

OTHERS: J. Robinson
R. Emde

Research Psychologist, IBG
Professor

Univ. Colorado
Univ. Colorado

COOPERATING UNITS (if any)

Institute for Behavior Genetics, University of Colorado
Health Sciences Center, University of Colorado

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.55

PROFESSIONAL:

.15

OTHER:

.40

CHECK APPROPRIATE BOXES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A behavior genetics research design is used to examine early precursors of adjustment and maladaptive behavior patterns in monozygotic and dizygotic twins. The twins are studied from late infancy through middle childhood in a collaborative study based in Colorado. The purpose is to assess genetic change and continuity in temperament, emotional organization, and cognition. Children are observed in structured and naturalistic situations in the home and laboratory. The specific goals of the NIMH component of the study are (1) to describe early development of prosocial and anti-social orientations, (2) to identify affective components that underlie these behaviors, and (3) to estimate the extent to which these early patterns of social-emotional development are heritable. Both mz and dz twins show highly synchronized, coordinated patterns of social interaction with their twin partners. They are equally prosocial and empathic to individuals outside the dyad, but patterns of generalization from the dyad to others differ for mz and dz twins. Empathy and guilt increase in frequency during the second year of life, while showing a decrease in genetic influence during this time period. In contrast, fear and anger are present earlier and show stronger evidence for heritability. Gender differences are present, particularly for empathy which is more apparent in girls.

Project Description

This work focuses on (1) the early development of individual differences in prosocial and anti-social orientations in children and (2) the emotions (e.g. empathy, guilt, fear, and anger) that accompany those behavior patterns. These dimensions of social-emotional functioning are studied in monozygotic and dizygotic twins, beginning in late infancy and through middle-childhood. The purpose is to assess early genetic influence that may contribute to patterns of social adjustment and maladaptation observed in later periods when children make a transition to pre-school and school settings. Assessment procedures developed at NIMH are applied to a large sample of mz and dz twins in Colorado, studied at the Institute for Behavior Genetics in Boulder. The NIMH component of the work on social-emotional development is part of a larger collaborative investigation funded by the MacArthur Foundation, on genetic change and continuity in temperament, emotional organization and cognition in identical and fraternal twins.

Methods Employed and Major Findings

Three-hundred and fifty pairs of monozygotic and dizygotic twins are seen in both home and laboratory settings at 14 months, 20 months, 24 months, 36 months, 48 months, and 52 months of age. A variety of procedures are used to assess different aspects of temperament, emotional organization and cognition. Within the social-emotional domain, prosocial patterns in distress and non-distress situations are observed in multiple occasions (empathic concern, cooperation, sharing, help). The quality of twins social interactions with each other is evaluated, as well as their prosocial orientations to the caregiver, examiner, and an infant in distress. Negative emotions such as anger and anxiety are observed under structured conditions designed to emotionally challenge children. Caregiver reports of children's empathy, guilt, fear, anger, and other negative emotions also are obtained.

Current heritability estimates and analyses of developmental patterns are based on approximately 100 pairs each of mz and dz twins at 14, 20 and 24 months of age. Both mz and dz twins show highly synchronized, coordinated patterns of social interaction with their twin partners, and they are equally responsive to individuals outside the dyad. Patterns of generalization from the dyadic relationship to other individuals differ, however, for mz and dz twins. Empathic, prosocial patterns and guilt become increasingly frequent during the second year of life, similarly for mz and dz twins. Evidence for genetic influence is modest for these 'social' emotions and diminishes in strength over this time period. In contrast, there is strong evidence for heritability of anger and fear, that continues throughout the second year of life. Fear and anger are emotions that occur earlier in development and may be less subject to socialization influences.

Proposed Course

Data collection is ongoing and will continue for several years. Currently follow-up assessments of the children at 7 years of age and beyond are being planned. The goals are (1) to continue to examine genetic continuity and change over time in emotional organization, temperament and cognition and (2) to assess how these factors predict quality of peer relations, psycho-social functioning and behavior problems following transition and adjustment to school. Preparation of articles for publication continues.

Significance to Biomedical Research and the Institute Program

Prosocial and anti-social orientations, and the emotions that accompany them, emerge early in development. As children grow older, these aspects of social-emotional functioning come to be part of more broadly based patterns that, in turn, can represent important variations in competence and adjustment vs. dysfunction and maladjustment in social relationships. This includes, for example, the capacity vs. inability to share, cooperate, negotiate, nurture; or the tendency to deceive, harm, and

exploit others vs. the ability to refrain from harm-doing. Because such difficulties in relationships are known to predict later adjustment and psychiatric problems, it becomes important to study early

dispositional characteristics, as well as environmental processes, that contribute to these variations amongst individuals.

Publications

Emde, RN, Plomin, R, Robinson, J, Resnick, JS, Campos, J, Corley, R, De Fries, J, Fulker, DW, Kagan, J, and Zahn-Waxler, C. Temperament, emotion and cognition at 14 months: The MacArthur Longitudinal Twin Study. Child Development, in press.

Zahn-Waxler, C and Smith, KD. The development of prosocial behavior. Handbook of social development: A life-span perspective. New York: Plenum Press, in press.

Zahn-Waxler, C. The case for empathy: A developmental perspective. Psychological Inquiry, 1991, 155-158.

Zahn-Waxler, C, Robinson, J, and Emde, RN. The development of empathy in twins. Developmental Psychology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02448-04 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Prediction of Conduct Problems During the Transition from Preschool to School Age

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	P. Cole	Senior Staff Fellow	LDP NIMH
CO-PI:	C. Zahn-Waxler	Chief, Section on Child Behavior Disorders	LDP NIMH
OTHERS:	F. Putnam	Medical Officer	LDP NIMH
	N. Fox	Professor	U. of Maryland
	G. Municchi	Foreign Visiting Fellow	LDP NIMH
	M. Linnoila	Scientific Director	LCS NIAAA
	G. Brown	Clinical Director	LCS NIAAA
	S. Suomi	Chief	LCE NICHD

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

Section on Child Behavior Disorders

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.55

PROFESSIONAL:

.75

OTHER:

2.80

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is the identification of predictors of stable conduct problems in young children. Existing research indicates that antisocial patterns in youth and adults are associated with a history of early childhood behavior problems, but prospective research indicates that only some difficult young children develop antisocial patterns and related psychiatric disorders. This study follows a sample of children who vary in the level of preschool age behavior problems they demonstrate, assessing the children during the preschool years (ages 4-5) and again during the completion of the first year of elementary school (ages 6-7). Dispositional and family correlates of antisocial patterns, including social, personality, cognitive and emotional developmental characteristics, are assessed. Observational, psychophysiological, biochemical, cognitive, and sociocognitive data are collected. The findings will yield information for (1) profiling children at-risk for chronic behavior problems, and (2) identifying factors which deter or promote continuity of problems.

Project Description

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Methods and Findings

Approximately 80 preschool age children and their parents were recruited through newspaper announcements and flyers sent to child care centers in the greater Washington area; some notices targeted hard-to-manage preschoolers and the rest recruited all four-year-olds. Children were then classified in regard to behavioral risk for conduct problems using standardized behavioral rating scales completed independently by mothers and teachers. The classification yields three approximately equal groups of children at low, moderate, and high risk.

Time 1 data collection is completed with the exception of one session for one family. Data analyses are underway. Dr. Cole's work focuses on (a) examination of individual differences in children's regulation of emotion and (b) the potential role of cognitive processes, called executive function, in relation to children's behavioral control. Dr. Zahn-Waxler's work focuses on (a) adaptive and maladaptive socialization patterns and (b) disturbances in internal representations (social cognitions) and empathic, prosocial development.

Time 2 data collection is currently underway. Approximately 2/3 of the sample are now in first-grade. Time 2 data collection involves 3 visits in which dispositional and familial constructs are assessed using observational, sociocognitive, and questionnaire data. At this time, standardized psychiatric diagnostic interviews of the children, using the Diagnostic Interview for Children, Parent and Child versions, are being conducted (in collaboration with Drs. Linnoila and Brown, NIAAA). School adjustment is also assessed using teacher ratings of social skills and standardized behavior rating scales. The remaining 1/3 of the sample will be followed in the 1992-1993 academic year.

These longitudinal data will be used to generate models to predict stability of behavioral problems during the transition from preschool to school age, using a developmental psychopathology conceptual approach. In particular, we will examine characteristics of the child, as assessed by a variety of different measures, and characteristics of the child's familial situation, also measured by a variety of means, to examine the degree to which these sets of factors contribute to the prediction of the child's psychiatric status and level of behavioral difficulty in elementary school.

Significance to Biomedical Research and to the Program of the Institute

Scientific information on the etiology of conduct disorders, particularly in terms of early childhood development, is a priority area in the NIMH National Plan. It is both a scientific and a practical problem that we do not know which young children are "going through a phase" or developing a little more slowly, and which young children are on a trajectory of stable behavioral difficulties. This study

addresses problems of evaluation and prediction by contributing to the construction of a possible profile of risk for stable behavior problems and therefore the prevention of conduct disorder, oppositional defiant disorder, and antisocial personality disorder.

Proposed Course

Time 2 data collection will continue through 1993, the year in which the second group of children are completing first grade. Subsequently, new protocols are likely addressing both a third follow-up of the children, and the development of a sample to examine prevention strategies based on the findings from the prediction study.

Related publications

Cole, P.M., & Putnam, F.W. (1992). Effects of incest on self and social functioning: A developmental psychopathology perspective. Journal of Consulting and Clinical Psychology, 60, 174-184.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

201 MH 02449-04 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Long-term Effects of Father-Daughter Incest

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: P. Cole

Senior Staff Fellow

LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Project terminated. This is a final report.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02487-03 LDP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Attachment Relationships and Maternal Affects in High Risk Families		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	E. DeMulder	Staff Fellow LDP NIMH
CO-PI:	M. Radke-Yarrow	Chief LDP NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.30	.15	.15
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The objective of the present study is to examine the <u>psychosocial contexts of attachment relationships</u> in families with <u>unipolar depressed, bipolar depressed and well mothers</u>. Since children of emotionally disturbed parents are at high risk for developing emotional and behavioral problems, this high-risk sample of affectively ill mothers and their children provides an opportunity to answer important questions about the organization of normal and atypical behavior. The aim of the research is to identify factors that put children at <u>risk for psychosocial disorders</u> and, further, to understand the <u>processes and mechanisms</u> by which these factors operate.</p> <p>Attachment assessments and behavioral observations are made as part of the longitudinal study (see Annual Report MH 02144). Analysis of these data revealed that mothers with bipolar illness are more likely than well mothers to have children who are insecurely attached. Affective behavior expressed by both mother and child was strongly related to attachment security. For example, there is a striking link between mother's downcast negative mood and insecure attachment, but this is only true for depressed mothers and their children.</p> <p>This is a final report.</p>		

Project Description:

The objective of the present study is to examine the psychosocial contexts of early attachment relationships in families with unipolar depressed, bipolar depressed and well mothers. To understand the processes involved in the development and maintenance of the attachment relationship, it is essential that salient attachment-related dimensions of the mother-child relationship are identified and examined. The study of interaction patterns between mother and child is of primary importance in linking socio-emotional aspects of the mother/child relationship and to assess their significance in terms of risk and protective factors in the child's development. The goal of such research is to go beyond identification of factors that put children at risk to understanding the processes and mechanisms by which these factors lead to the emergence of psychosocial disorder.

Methods and Findings:

The source of data is the longitudinal study of unipolar depressed, bipolar depressed and well mothers (MH 02144). Attachment assessments were made (based on the child's pattern of behavior to the mother after a brief separation) when the children were between 1 1/2 and 3 1/2 years of age. Videotaped records of behavior in the naturalistic setting were coded minute by minute for the dominant affect expressed. The tapes were coded for both mother and child when they were alone together, approximately 5 hours.

Examination of the data revealed that bipolar depressed mothers and their children were more likely to have insecure attachment relationships than were well mothers and their children. There were no differences in the proportions of secure and insecure children of unipolar depressed and well mothers.

Mother and child affective behavior was related to the quality of the attachment relationship. Mothers' downcast mood was highly related to insecure attachment. This relation was stronger for depressed mothers than for well mothers and stronger for daughters than for sons. In addition, more mothers of insecure children expressed a high frequency of anger/irritability and a high frequency of multiple negative affects (particularly in the depressed-mother groups) than did mothers of secure children. In the bipolar-mother dyads, insecure children were more anxious than were secure children. Insecure mothers and their children expressed less tenderness/affection to each other than did secure dyads and their affect was less often neutral pleasant.

We have now examined further the psychosocial contexts of these at-risk attachment relationships with an analysis of disciplinary patterns and child noncompliance. We have found, again, that links to attachment are different for well and depressed-mother groups and different for girls and boys. For instance, secure girls were the most likely to comply with their mothers immediately while secure boys were the least likely to do so. When control attempts were resisted, well mothers and their secure children were more likely to resolve the issue through compromise than were well mothers and their insecure children (consistent with the attachment concept of a secure 'goal corrected partnership'). This was not the case in the depressed mother groups. In addition, secure girls and their mothers were more likely to compromise than any other group.

Further analyses entailed a closer examination of the different patterns of insecure attachment in relation to the relationship dimensions considered. Children who showed insecure-ambivalent attachment patterns expressed more sadness (crying, and so forth) than any other attachment group, consistent with previous evidence that these children may be more prone to distress. Children with insecure-disorganized patterns of attachment (who are considered the most highly insecure) were more downcast than were secure

children. The mothers of these children showed more downcast and less neutral/pleasant affect, were the most likely to show high levels of more than one negative affect, and were the least likely to express tenderness and affection toward their children. This study provides a fuller understanding of early risk and protective factors in the development of psychopathology.

Significance to Biomedical Research:

There is now extensive evidence that the children of psychiatrically disturbed parents are themselves at high risk of developing emotional and/or behavioral problems. This study aims to identify aspects of the mother/child relationship that are 'carried forward', those that may serve as protective factors in the child's development, and more importantly, those that may serve to render the child vulnerable to psychopathology.

Proposed Course:

This is a final report.

Publications:

DeMulder, E.K. & Radke-Yarrow, M. (1991). Attachment with affectively ill and well mothers: concurrent behavioral correlates. *Development and Psychopathology*, 3, 227-242.

DeMulder, E.K., Radke-Yarrow, M., Nottelmann E., & Belmont B. Attachment relationships in high risk families: the role of affect. Presented at the Biennial Conference of the Society for Research in Child Development, Seattle, April 1991.

Radke-Yarrow M. (1991). Attachment patterns in children of depressed mothers. In: Stevenson-Hinde J, Parkes CM, eds. Attachment through the life cycle. London: Routledge.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02488-03 LDP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Parent-Child Relationships in Late Childhood and Early Adolescence		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	L. Tarullo	Staff Fellow LDP NIMH
CO-PI:	E. DeMulder	Staff Fellow LDP NIMH
	P. Martinez	Medical Staff Fellow LDP NIMH
	M. Radke-Yarrow	Chief LDP NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.20	.80	.40
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In the longitudinal study of children of <u>affectively ill parents</u> and well parents, parent-child relationships have been evaluated in early childhood (MH 02487 and MH 02409). In this study, mother-child interaction and child perceptions of maternal support were assessed for two siblings, ages 8-11 years and 12-16 years. The objective of the study is to examine <u>affective/communicative patterns</u> , comparing affectively ill and well mother-child dyads: a) Are there particular patterns of interaction more common in dyads with mothers who have affective illnesses? b) Do these mother-child patterns of interaction differ for children at different developmental periods and of different gender? c) Do these patterns differ with children who have or do not have psychiatric disorders themselves? d) Is maternal current psychiatric status linked more closely with <u>dysfunctional interaction</u> patterns than is maternal lifetime diagnosis? Relational difficulties, observed in interaction and revealed through child representation, were linked to both maternal affective illness and <u>child psychiatric status</u> . The results illuminate interactive processes through which psychopathology may be perpetuated in families. This is a final report.		

Project Description:

In the longitudinal study of children of affectively ill and well parents, parent-child relationships have been evaluated in early childhood (MH 02487 and MH 02409). Children of parents with affective disorders are at risk for the development of psychopathology; symptoms of depressive illness are typically manifested as children move into adolescence. Assessing the quality of the relationship between depressed parents and their children during this crucial transition contributes to our understanding of the processes involved. Observational assessment of semi-structured interaction between parent and child, combined with the child's reports concerning parental support, provide multiple perspectives on the nature and quality of parent-child communication, investment, and affective climate.

Methods and Findings:

Eighty-eight families, participants in the longitudinal study of families with well and depressed parents, are observed when the younger of the two siblings is 8 to 11 years of age, and the older sibling is 12 to 16 years of age. Each parent-child dyad is given questions to ask each other about their relationship (e.g., How well do you think I know what you like and are like? How are you and I alike and different?), which serve as the basis for an informal discussion lasting ten to fifteen minutes. Sessions are videotaped through a one-way mirror. This unique context allows observation of the relationship in action as it is being discussed. Qualitative aspects of interaction are assessed, including participants' communication skills, level of investment in the relationship, identification, self-reflection, empathy, and affective tone.

Analyses of mother-child dyads have been completed. Principal components analysis yielded six factors describing mother behavior: mother attuned/open to discussion; mother critical/irritable; mother shows positive outlook/happy mood; mother praises child; mother passive/acquiescent; mother affectionate/warm. The five factors describing child behavior were: child attuned/open to discussion; child comfortable/happy mood; child critical/irritable; child affectionate/warm; and child sad/negative outlook. Two factors describing dyadic qualities were: dyad harmonious/warm; and dyad invested/open to discussion.

A related measure, taken at the same developmental period, is a representation of each child's satisfaction with parental support in the domains of emotional, instrumental, and informational help and companionship, as well as their perceptions of the level of conflict in the relationship.

Analyses focused on the links between mother-child interaction and maternal affective illness (both type and recency of episodes), child current disorder, child developmental period, and child gender. Relational difficulties, observed in interaction and revealed through child representation, were linked to both maternal affective illness and child psychiatric status. The findings concerning maternal lifetime diagnosis and maternal current psychiatric status revealed different patterns of relations. However, in general, recency of maternal episode was not linked more strongly to problems in interaction than was lifetime diagnosis.

Preadolescent dyads with affectively ill mothers were less invested and open to discussion than were dyads with well mothers. Mothers and their preadolescents were more critical/irritable with each other and less harmonious warm in interaction when the child had a psychiatric disorder. With adolescents, affectively ill mothers were more critical/irritable than were well mothers. While preadolescents were more comfortable/happy with bipolar mothers, adolescents were more comfortable/happy with unipolar mothers. Gender differences were apparent, particularly in regard to mother's current psychiatric status. Preadolescent daughters reported more conflict with mothers who had been in episode within the month, and interactions in adolescent daughter dyads were more critical and less harmonious with such mothers.

The results illuminate interactive processes through which psychopathology may be perpetuated in families.

Significance to Biomedical Research:

Assessing the relational context experienced by children at risk for the development of affective disorders contributes to our understanding of the environmental factors which may contribute to maladaptive outcomes for such children.

Related Publications:

Tarullo, L.B., DeMulder, E.K., Martinez, P.E., & Radke-Yarrow, M. Dialogues with preadolescents and adolescents: Mother-child interaction patterns in affectively ill and well dyads. Submitted for publication.

Proposed Course:

This is a final report.

Project Description:

A child's ability to initiate and maintain positive and competent interaction with peers is an important developmental task of childhood. In this study, we have examined how children's temperament and maternal rearing practices are related to children's social competence and modes of interaction with peers.

Methods and Findings:

Assessments were made in toddlerhood of the child's shyness and behavioral inhibition. When the children were seen at age 5, these characteristics were again assessed. Each child was observed with an unfamiliar peer of same sex and age in a standard situation of getting acquainted and playing with each other.

Interactive behaviors (approach/withdrawal, verbal overtures to peer, other affect expression), more general patterns of play (e.g., solitary, parallel, group), social passivity, aggressive and prosocial acts were coded. At both periods of development, maternal techniques of regulating child behavior were also coded.

Inhibition to unfamiliar persons at age 2 predicted a highly withdrawn and passive pattern of behavior with peers at 5. Early inhibition to unfamiliar environment predicted decreased involvement in group play. Analysis of the changing dynamics of the ongoing peer interaction revealed that the role of child inhibition as a predictor of social behavior may be mostly evident during the initial encounter with the peer. Children who as toddlers were particularly socially inhibited during the initial phase of peer interaction showed significantly stronger patterns of socially passive and withdrawn behavior. Associations were found between maternal socialization practices and children's interactions with peers. Mothers' power-assertion and clarity while disciplining their children, assessed when children were toddlers and contemporaneously, in independent contexts, were associated with the qualities of children's influence styles towards peers. Maternal non-over-assertive but clear regulatory practices were associated with children's competent styles with peers, whereas power-assertion and lack of clarity were related to less competent influence styles with peers. Children who use abrasive strategies of influence with their mothers were aggressive and unsuccessful in influencing their peers.

Significance for Biomedical Research:

Disturbed and incompetent patterns of children's interactions with peers are among the most often identified risk factors and concomitants of developmental maladaptions. This study adds to our understanding of the contribution of temperament and parental regulatory practices to the child's adaptive or maladaptive behavior with peers.

Proposed Course:

This is a final report

Publications:

Kochanska G., & Radke-Yarrow (1992). Inhibition in toddlerhood and the dynamics of the child's interaction with an unfamiliar peer at age five. Child Development, 63, 325-335.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02490-03 LDP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Predictions from Early Childhood to Later Psychosocial Functioning		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. Radke-Yarrow	Chief LDP NIMH
CO-PI:	P. Martinez	Senior Staff Fellow LDP NIMH
OTHERS:	E. DeMulder	Staff Fellow LDP NIMH
	K. McKann	Special Vol. LDP NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.15	1.00	.15
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The research objective was to follow the development of securely and insecurely attached children whose mothers were clinically <u>depressed</u> and mothers who were without psychiatric disorders. Depressive symptomatology involves impairments in domains of maternal functioning that, in <u>attachment</u> theory, are especially influenced by attachment: sense of self as unworthy, difficulties in engaging with the environment, dysregulated affect, negative expectations of others, and difficulties in developing positive, committed relationships. The research question is: how do secure and insecure attachments, relationships with depressed and well mothers, carry forward in <u>children's psychosocial development</u> ? One hundred mother-child pairs, 37 normal control mothers, and 63 depressed mothers were studied. Attachment and family stressors were assessed when the children were preschool age. Children's psychiatric status was evaluated 3 years and 6 years later. No main effects were found for attachment. However, attachment exercises more subtle effects on child outcome in interaction with other family variables. This is a final report.		

Project Description:

The research objective was to follow the development of securely and insecurely attached children whose mothers were clinically depressed and mothers who were without psychiatric disorders. Depressive symptomatology involves impairments in domains of maternal functioning that, in attachment theory, are especially influenced by attachment: sense of self as unworthy, difficulties in engaging with the environment, dysregulated affect, negative expectations of others, and difficulties in developing positive, committed relationships. The research question is: how do secure and insecure attachments relationships with depressed and well mothers, carry forward in children's psychosocial development?

Methods and Findings:

One hundred mother-child pairs, 37 normal control mothers, and 63 depressed mothers were studied. Attachment was measured when the children were preschool age. Family stressors were also measured at that time. Follow-up psychiatric evaluations of the children were made 3 and 6 years later.

There were no main-effects of attachment, on children's later psychosocial development. Attachment exercises more subtle effects on child outcome in interaction with or in the context of, other variables in the child's early experiences. Secure attachment to unipolar depressed mothers was significantly associated with children's depressive affect at 5 to 6 years of age. Children who suffered the loss of a significant person in their first three years showed significantly more depressive affect and anxieties at beginning school age. Also, children with insecure attachments and in family circumstances of high marital discord were more likely to manifest depressive affect at 5 to 6 years of age.

Significance to Biomedical Research:

Knowledge of precursors of later problems provides information on developmental processes. Such information is relevant for prevention and intervention.

Proposed Course:

A manuscript is being submitted for publication
This is a final report

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02491-03 LDP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Development of Offspring of Affectively Ill and Well Parents		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	M. Radke-Yarrow	Chief LDP NIMH
Co-PI:	P. Martinez	Senior Staff Fellow LDP NIMH
OTHERS:	J. Richters Chief, Conduct Disorders Program CADRB	
COOPERATING UNITS (if any) CADRB		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.75	.75	1.00
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This is a <u>longitudinal study of children of affectively ill and well parents</u> , from early childhood to adolescence. The research objectives are: (a) to follow the course of children's <u>psychosocial development</u> , and (b) to investigate the relation of parents' diagnoses, family history, and family functioning and family stress factors to children's functioning. Assessment of child functioning includes psychiatric interviews with the children and their parents, other parent and child interviews. Models for longitudinal assessments have been worked out. Analyses of the data are well underway.		

Project Description:

This is a longitudinal study of children of affectively ill and well parents, from early childhood to adolescence. The research objectives are: (a) to follow the course of children's psychosocial development and (b) to investigate the relation of parents' diagnoses, family history, and family functioning and family stress factors to children's functioning. Mothers' and fathers' diagnoses are bipolar illness, unipolar depression, well controls (SADS-L & SCID). Within diagnoses, there are further classifications on severity of the illness. Assessment of child functioning includes psychiatric interviews with the children and their parents, other parent and child interviews and systematic observations of behavior. Children's problems are evaluated in terms of DSM III R categories, as well as concepts derived from developmental psychology: self control, emotional regulation, social relationships, self concepts, competencies, and cognitive functioning.

Methods and Findings:

Assessments of 107 families covering the span of childhood have been completed. Models for longitudinal assessments have been worked out. Analyses of the data are well underway.

Significance to Biomedical Research:

Unipolar and bipolar affective illness are major mental health problems. Understanding the individual developmental histories of affective problems that become "full blown" in adolescence and adulthood is essential for an understanding of etiology; it is also important for prevention and intervention.

Proposed Course:

These analyses address a core set of issues in the longitudinal study and will be either a scientific monograph or several substantial papers. These analyses make use mainly of standard instruments of individual assessment. Following this monograph, a second analysis will be directed to integrating the more intensive, individual analyses of development.

Publications:

Young Children of Affectively Ill Parents: A Longitudinal Study of Psychosocial Development, J. Am. Acad. Child Adolesc. Psychiatry, 31:1, January 1992

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02493-03 LDP																				
PERIOD COVERED October 1, 1991 through September 30, 1992																						
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effects on Children of Exposure to Chronic Community Violence																						
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI:</td> <td style="width: 30%;">P. Martinez</td> <td style="width: 30%;">Senior Staff Fellow</td> <td style="width: 10%;">LDP NIMH</td> </tr> <tr> <td>CO-PI:</td> <td>J. Richters</td> <td>Chief, Conduct Disorders Program</td> <td>CADRB NIMH</td> </tr> <tr> <td>OTHERS:</td> <td>G. Municchi</td> <td>Visiting Staff Fellow</td> <td>LDP NIMH</td> </tr> <tr> <td></td> <td>D. Rubinow</td> <td>Clinical Director</td> <td>NIMH</td> </tr> <tr> <td></td> <td>M. Radke-Yarrow</td> <td>Chief</td> <td>LDP NIMH</td> </tr> </table>			PI:	P. Martinez	Senior Staff Fellow	LDP NIMH	CO-PI:	J. Richters	Chief, Conduct Disorders Program	CADRB NIMH	OTHERS:	G. Municchi	Visiting Staff Fellow	LDP NIMH		D. Rubinow	Clinical Director	NIMH		M. Radke-Yarrow	Chief	LDP NIMH
PI:	P. Martinez	Senior Staff Fellow	LDP NIMH																			
CO-PI:	J. Richters	Chief, Conduct Disorders Program	CADRB NIMH																			
OTHERS:	G. Municchi	Visiting Staff Fellow	LDP NIMH																			
	D. Rubinow	Clinical Director	NIMH																			
	M. Radke-Yarrow	Chief	LDP NIMH																			
COOPERATING UNITS (if any) Child and Adolescence Disorders Research Branch, NIMH																						
LAB/BRANCH Laboratory of Developmental Psychology																						
SECTION																						
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892																						
TOTAL STAFF YEARS: .50	PROFESSIONAL: .25	OTHER: .25																				
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews																						
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p> This study is an effort to learn about the nature and correlates of children's exposure to <u>chronic community violence</u>. What is the extent to which young children living in chronically violent <u>inner-city environments</u> are exposed, both directly and indirectly, to various forms of violence? What are the effects of these exposure patterns on children's psychiatric symptomatology, health, and social, emotional, and cognitive functioning? How are exposure and its consequences -- both adaptive and maladaptive-- mediated by characteristics of the children, their families, and communities. </p>																						

Project Description:

The study is an effort to learn about the nature and correlates of children's exposure to chronic community violence. What is the extent to which young children living in chronically violent inner-city environments are exposed, both directly and indirectly, to various forms of violence? What are the effects of these exposure patterns on children's psychiatric symptomatology, health, and social, emotional, and cognitive functioning? How are the exposure and its consequences-- both adaptive and maladaptive -- mediated by characteristics of the children, their families, and communities.

Methods and Findings:

The study is divided into two phases. During phase 1, data concerning children's violence exposure, social emotional adjustment and health, is solicited from mothers and teachers of children attending grades 1, 2, 5, and 6, of an elementary school located in a moderately violent area of Washington, D.C. This provides information about the range of violent experiences to which the children have been exposed, as well as an estimate of the extent to which exposure patterns are associated with symptoms and behavior problems.

Phase 2 consists of intensive assessment of approximately 30 children who have been exposed (as determined by Phase 1 data) to low, moderate, and high levels of violence, and who are matched for to the extent possible on relevant family and neighborhood characteristics. Assessments will include (1) standardized psychiatric interviews with the children and their mothers (mothers will be interviewed regarding their own and their children's symptoms; children will be interviewed only concerning their own symptoms), (2) standardized and exploratory interviews/procedures with children and their mothers concerning their thoughts and emotions related to community violence and (3) measurement of salivary cortisol, as a biological measure of stress, in both the child and the mother.

During phase 1, 165 children attending grades 1, 2, 5, and 6 were seen at the school; data concerning distress symptoms and violence exposure were collected independently from mothers and their children. Parent's and children reports indicate that a significant number of children had been victimized by violence in the community; but children were significantly (between two and four times) more likely to have witnessed moderate and severe community violence than to be victimized directly. Forms of violence witnessed by children ranged from witnessing drug deals and muggings, to stabbings, shootings, and murders. Agreement between parents and their son's about community violence exposure was moderate and significant for both younger and older boys but not for girls. The majority of violence experienced by older children took place near their homes and involved persons familiar to them (i.e. family members, friends and acquaintances). The prevalence of within-family violence between adults was relatively common; rates of minor and severe family violence reported by parents were between five and six times the national average.

Mother's ratings of children's distress symptoms were significantly related to younger and older boy's self-ratings of distress; mother's ratings were not, however, significantly related to their daughter's self-ratings of distress. Mothers also tended to significantly underestimate the extent to which their children were experiencing distress symptoms, including depression, fears, anxieties, intrusive thoughts, worrying about their safety, and sleep problems. Child-reported violence was significantly related to distress symptoms in both older and younger children.

Proposed Course:

The follow up is being completed.

Significance to Biomedical Research:

Despite the high frequency of community violence in urban centers, there has been little systematic research into the nature and consequences of children's exposure to chronic violence. Clinical-descriptive reports indicate that children's reactions include intrusive thoughts, fear of recurrence, anxieties, difficulty concentrating, depression, psychosomatic disturbances, sleep disturbances, and other symptoms that are associated with post-traumatic stress disorder in adults. Large numbers of children are at risk of serious mental health problems in the immediate and long term sense.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02499-03 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Development of Problem Aggression in Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal investigator.) (Name, title, laboratory, and institute affiliation)

PI: C. Zahn-Waxler Chief, Section on LDP NIMH
Child Behavior Disorders
OTHER: P. Cole Senior Staff Fellow LDP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.60

PROFESSIONAL:

.30

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patterns of emotion, behavior, and social-cognition in both hypothetical and real situations of interpersonal conflict and distress are examined in aggressive, difficult-to-manage preschool children. Anti-social children are commonly described as deficient in moral reasoning, unempathic, and unlikely to engage in prosocial acts (e.g., sharing, help, cooperation, negotiation). This study investigates individual differences in the organization of these social patterns in children at risk, to better understand dimensions that underlie the development of serious interpersonal aggression and ruleviolation. The first wave of assessment has been completed and data isbeing analyzed for approximately 80 children at low, moderate, or high risk for later conduct problems. Children at risk make fewer prosocial choices in experimental situations, though problem girls construct more prosocial solutions than problem boys. Even the most aggressive children do not show diminished empathic, prosocial behaviors at this age, toward adults in distress. These children are more emotionally aroused, however, which may interfere with later empathic development and generalized patterns of caring for others.

Project Description

Children who display behavior problems early in development are at known risk for psychiatric disorders and pervasive problems in social relationships. This study examines the early organization of one set of risk factors thought to be implicated in the development of anti-social patterns and conduct problems. Specifically, the focus is on disturbances in the expression of social skills that involve internalization of positive regard for others. We examine whether young children with behavior problems show atypical patterns in empathic expressions, interpretation of affective cues, prosocial behaviors, and autonomic arousal during mood induction. Linkages across these domains also are compared in children with and without behavior problems.

Methods Employed and Major Findings

Approximately 80, 4-5-year-old children at low, moderate, or high risk for conduct problems are seen in a series of laboratory sessions (# Z01 MH 02448-03). Several procedures embedded in these sessions assess children's perceptions, cognitions, emotions and behaviors in hypothetical and real situations of interpersonal conflict and distress. The following measures are obtained: (1) forced choice, self-reports of emotions and behavioral strategies in structured scenarios (2) narratives and symbolic play reflecting internal representations of conflict and distress, (3) empathic and prosocial behaviors in response to others' (simulated) distresses, (4) affective arousal indexed by facial and vocal expression, heart rate, and skin conductance during a mood induction procedure, (5) self-reports of emotion following mood induction, (6) observations of behavioral compliance with interpersonal and situational requirements.

Data collection has been completed for this phase of the project and data are in varied stages of coding and analysis. Children independently identified as having behavior problems based on the Achenbach Child Behavior Check List show more aggressive solutions and fewer prosocial choices when presented with hypothetical interpersonal dilemmas, suggesting that they are cognitively "primed" for hostile, more than solution-oriented ways of coping. Problem girls endorse more prosocial solutions than boys, a factor that may be linked to the later low levels of anti-social patterns that typically characterize girls.

Children's responses to (simulations of) distress in mothers and examiners revealed few group differences. Even the most aggressive children (all boys) show empathic concern and levels of prosocial behavior comparable to the control group. Aggressive, anti-social children, then, do not necessarily begin life with a muted capacity for concern for others. The callous indifference often observed in children with behavior problems may take considerable time to develop. The most aggressive children, in fact, showed the most emotional arousal in response to their mothers distress and sometimes showed 'precocious' levels of caregiving. High levels of emotional overinvolvement reflect personal distress, in contrast to sympathetic distress where the emotional concern is outwardly focussed on the victim. We will examine whether this distinction between personal distress and sympathetic concern is also evidenced in different types of heart rate patterns. The high levels of emotionality shown by these children in actual distress encounters, in conjunction with their focus on aggressive rather than prosocial solutions to interpersonal problems in symbolic representations, portends a maladaptive developmental trajectory for many of them.

Proposed Course

Upon completion of data analysis a manuscript will be prepared for publication. Later, factor analyses will be conducted with the individual measures to determine which dimensions should be retained for longitudinal analyses.

Significance to Biomedical Research and the Institute Program

An aim of prevention research is to identify early in development characteristics of the child and the environment that contribute to later problem behavior. If processes contributing to behavior problems

can be identified in the early years of life, more effective intervention procedures could be planned. Emphasis here is on identifying aspects of social competence that are prerequisites for mature interpersonal relationships. Prosocial skills such as the ability to help, share, cooperate, negotiate and care for others may mitigate against problem aggression.

Publications

Cummings, EM, Zahn-Waxler, C. Emotions and the socialization of aggression: Adults' angry behavior and children's arousal and aggression. A Fraczek and H Zumkley, eds. Socialization and Aggression, New York and Heidelberg: Springer-Verlag, in press.

Zahn-Waxler, C, Radke-Yarrow, M, Wagner, E and Chapman, M. Development of concern for others. Developmental Psychology, 1992, 28 (1), 126-136.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 MH 02559-02 LDP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Children's Views about Conflict as a Function of Gender and Maternal Depression		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI: C Zahn-Waxler OTHERS: D. Hay E. Cummings R. Iannotti S. Denham	Chief, Section on Professor Professor Professor Professor	LDP NIMH MRC Child Psychiatry University of West Virginia Georgetown University George Mason University
COOPERATING UNITS (if any) MRC Child Psychiatry Unit, London Georgetown University University of West Virginia		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS: .20	PROFESSIONAL: .10	OTHER: .10
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Forty five-year-old children were interviewed about conflict with peers and also were observed in interactions with their playmates. Half of the children had a depressed caregiver, a condition known both to be associated with familial conflict and to place children at risk for <u>externalizing problems</u> , as well as <u>internalizing problems</u> . Associations between children's views about how conflicts are resolved and their actual behaviors in conflict situations were identified. Children who were observed to engage in more protracted conflicts with their friends, were also more likely to recommend <u>aggressive tactics in resolving conflict</u> in a hypothetical situation (mainly for children of depressed mothers). Young children, in general, showed sophisticated understanding of the distressing quality of disputes and they held systematic views about the nature of conflict. There were individual differences in their endorsements of tactics used to resolve conflict, that were primarily related to the child's gender and the mother's <u>affective disorder</u> . Sons of depressed mothers were very likely to recommend aggressive solutions to peer conflicts while the daughters virtually never endorsed aggressive solutions. Boys and girls of well mothers did not differ. These <u>exaggerations of normative sex role patterns</u> in children at risk, associated with parental affective disorder, may reflect the development of entrenched, stereotyped ways of coping that are precursors of internalizing vs. externalizing problems in females vs. males, respectively. This is a final report.		

Project Description

This study focuses on assessment of linkages between young children's understanding of conflict resolution and their abilities to resolve real disputes with playmates, in relation to a risk factor associated with conflictual familial experiences, i.e., parental depression. Based on more general proposals regarding familial conditions that might encourage a depressive orientation in females and an aggressive one in males, it was hypothesized that maternal depression would exaggerate normal sex differences between girls' and boys' views of conflict. More specifically, it was predicted that sons of depressed women would endorse highly aggressive tactics of conflict resolution while daughters would recommend highly socialized strategies.

Methods Employed and Major Findings

Forty, five-year-old children were interviewed about conflict with peers, during the course of observing a fight between two puppets. Children also were observed in interactions with their playmates. Half of the sample had a depressed caregiver, a familial condition known to place children at risk for externalizing, as well as internalizing problems. Associations between understandings of how conflicts are resolved and actual behaviors in conflict situations were identified. Children who were observed to engage in more protracted conflicts with their friends, were also more likely to recommend aggressive tactics in resolving conflict (mainly for the depressed sample). Young children, in general, showed sophisticated understanding of the distressing quality of disputes and they held systematic views about the nature of conflict. There were individual differences in their recommendations about tactics to be used in resolving conflict, that were primarily related to the child's gender and the mother's affective disorder. Sons of depressed mothers were very likely to recommend aggressive solutions to peer conflicts while the daughters virtually never endorsed aggressive solutions. Boys and girls of well mothers did not differ. Girls, in general, recommended more socialized tactics, regardless of whether or not the mother was depressed. Hence, only one of the two original hypotheses was borne out. Girls of depressed mothers did not show highly socialized patterns of conflict resolution. But their strategies were extremely non-aggressive, suggesting that in such families, girls may be suppressing their own anger and assertive, aggressive tendencies (resulting in helplessness), while boys are acting on them.

Proposed Course

The project has been completed. This is a final report.

Significance to Biomedical Research and the Program of the Institute

Social skills deficits, reflected both in children's understanding of the nature of disputes and their behaviors in conflict situations, may be precursors to later psychiatric problems and serious interpersonal difficulties. The present work demonstrates that when interview procedures pertaining to interpersonal conflict are sensitive to developmental level, it is possible to obtain detailed information about young children's understanding of conflict and attempts to resolve disputes. Early perturbations can thus be identified that may reflect risk for psychopathology in children too young to participate in the structured psychiatric interviews required for formal diagnostic assessments.

Publications

Denham S, Zahn-Waxler C, Cummings EM, Iannotti R. Social competence in young children's peer relations: Patterns of development and change. Child Psychiatry and Human Development, 1991, 22(1), 29-44.

Hay DF, Zahn-Waxler C, Cummings EM, Iannotti RJ. Young children's views about conflict with peers: A comparison of the daughters and sons of depressed and well women. Journal of Child Psychology and Psychiatry, in press.

Rubin KH, Both L, Zahn-Waxler C, Cummings EM, Wilkinson M. The dyadic play behaviors of children of well and depressed mothers. Development and Psychopathology, in press.

Zahn-Waxler C, Ridgeway D, Denham S, Usher B, Cole PM. Pictures of infants' emotions: A task for assessing mothers' and young children's verbal communications about affect. In: R Emde, J Osofsky, P Butterfield, eds., Parental perception of infant emotions. Clinical Infant Report Series. Washington, DC, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02560-02 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Individual Differences in Empathic Behavior in Children

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: M. Radke-Yarrow Chief LDP NIMH

CO-PI: C. Zahn-Waxler Chief, Section on Child Behavior Disorders LDP NIMH

OTHERS: P. Martinez Senior Staff Fellow LDP NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.85

PROFESSIONAL:

.25

OTHER:

.60

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK (Use standard unindented type. Do not exceed the space provided.)

This study inquires into young children's empathic behavior toward mother under conditions of high and low parenting risk (mother's psychiatric status), and in relation to child characteristics (gender, attachment, and psychiatric status). Observed interactions of mothers and their preschool age children were the source of data on empathy. Independent psychiatric assessments were made of mother (SADS) and child (play interview and parent report). Maternal diagnosis alone was not a strong predictor of children's empathy. Girls were more empathic than boys. Specific interacting factors of severe maternal depression, secure attachment, and child psychiatric problems were associated with the highest frequencies of children's empathic behavior. Empathy is discussed as an indicator of "health" or of "risk" in children of well and depressed mothers.

This is a final report.

Project Description:

Although symptoms of depression are not uniform across individuals and not all depressed mothers express all symptoms or express all symptoms in their maternal role, maternal depression is likely to expose children to repeated affective stress. What are the effects of such elevated exposure to emotional distress on the frequency of young children's empathic behavior and the processes underlying it?

Methods and Findings:

Ninety preschool-age children and their mothers, a subsample (based on age) of the NIMH longitudinal study were the participants. The affectively ill mothers (n=52) were classified on the severity-chronicity of their illness. Mothers and children interactions were observed and video-taped in a seminatural setting and in an experimental situation in which mothers were asked to simulate sadness.

The behavior of interest was the child's responses to mother's needs or emotional distress. Children's empathic behavior was analyzed in relation to mother's illness, child gender, child psychiatric problem, and the attachment relationship. Mother's diagnosis alone was not a significant predictor of children's empathy in naturalistic interaction, although children of depressed mothers responded more to mother's explicit display of sadness. Gender was a strong predictor. Girls were much more involved in mother's needs and stress than boys, regardless of mother's wellness or depression. Also, girls' concern about mother's sadness was equally strong in all maternal diagnostic categories. For boys, mothers' severe depression was necessary to bring out concerned responding to maternal sadness. Boys and girls not only differed in frequency of empathic behavior. This behavior was differently influenced by the attachment relationship. Secure attachment was associated in girls with more empathy, Regardless of mother's diagnosis, insecure attachment was associated in boys with more empathy. This further suggests possibly different processes underlying the empathy of boys and girls.

Differences in children's own emotional problems brought another level of interaction and meaning to the children's empathy. Offspring of severely depressed mothers who were securely attached to these ill mothers and who also had psychiatric problems were the subgroup exhibiting the most empathic interactions. This configuration can be interpreted as reflecting "enmeshment." The process of responding to the mother's needs has interfered with the child's own emotional integration.

Significance to Biomedical Research:

The ability to perceive and be responsive to affective communications from other persons is an essential for the child's psychologically healthy development.

Proposed Course:

A paper has been submitted for publication
This is a final report.

Publications:

Development of Concern for others, Developmental Psychology, 1992, Vol. 28, No.1, pp 126-136.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02561-02 LDP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cohesion in Families with Affectively Ill and Well Parents		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI	E. Nottelmann	Statistician LDP NIMH
CO-PI:	G. Germain	Research Psychologist LDP NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.70	.70	0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Family relationships</u> can function as <u>protective as well as risk factors in children's development</u> . In families with <u>parental affective illness</u> , the particular strengths and weaknesses of the family can mediate in the child's exchanges with his/her environment and, thus, maximize or minimize problems in the child. Family functioning is assessed using the Family Systems Test in which family relationships are depicted with figures on a grid-covered board: (a) from the perspective of each of two siblings and (b) from a perspective formed in consensus among family members. "Closeness", "orientation", and "power" are determined by distance between figures, directionality of the figures' faces, and number of "power blocks" under each figure, respectively, and <u>observer ratings</u> are made of family members' behavior while they work together constructing consensus representations. The ratings focus on behavior relevant to competencies and dysfunctions of the family as a unit, of the dyads within the family, and of individual family members. The <u>children's perceptions of family relationships</u> and qualities of <u>family and individual functioning</u> will be examined developmentally, incorporating measures from the larger project of which this study is a part.		

Project Description

Although parental affective illness places a child at risk for psychiatric and functional problems, the particular strengths and weaknesses of the family in which the child develops can mediate in the child's exchanges with his/her environment and, thus, maximize or minimize the appearance of problems. Family as well as interpersonal and intrapersonal functioning are assessed using the Family Systems Test in which family relationships are depicted with figures on a grid-covered board: (a) from the perspective of each of two siblings and (b) from a perspective formed in consensus among family members. "Closeness", "orientation", and "power" are determined by distance between figures, directionality of the figures' faces, and number of "power blocks" under each figure, respectively, and observer ratings are made of family members' behavior while they work together to construct family consensus representations. The specific aim of the project is to examine family and child functioning in terms of reported relationships and actual behavior observed within the family in addition to diagnostic status of the parent(s).

Methods and Findings

Mother, father, older sibling (11 to 15 years), and younger sibling (8 to 11 years) are studied in 37 normal control families, 21 families with a bipolar mother, and 49 families with a unipolar mother. Two approaches to examining family influences on child outcomes are used. Both involve the Family Systems Test in which family relationships are depicted with figures on a grid-covered board: (a) from the perspective of each of two siblings (procedure done individually) as well as (b) from a perspective formed in consensus among the parents and children. In each case, family relationships are represented by family members in three situations--typical, conflictual, and ideal.

The first assessment approach uses the construction of family relationships literally. In accord with instructions given by the experimenter, "closeness", "orientation", and "family decision-making power" are determined by distance between figures, directionality of the figures' faces, and number of power blocks under each figure, respectively. The second assessment approach consists of observer ratings of family members' behavior while they work together to construct the family consensus representations. The ratings focus on competencies and dysfunctions of the family as a unit, of the dyads within the family, and of individual family members, namely, the ability to coordinate and communicate effectively, the ability to maintain an affective climate in which there is appropriate expression of feelings and involvement, and use of power or influence (leadership, domination, and disruptiveness). Mothers and fathers are assessed in terms of how well they each fulfill two roles--the spousal and parental roles. The sibling relationship, too, is characterized. To further capture emotion-related problems, ratings are made of various affects and motivational or behavioral dispositions such as: happy affect, sadness, anxiety, anger, emotional lability, impulsivity, self-confidence, and involvement.

Formal data analysis has not yet begun. Preliminary analyses indicate sensitivity of the measures and variability in the functioning of the families. For example, the families varied widely in their ability to function in a coordinated and goal-directed manner. Major problems involved degree of "chaos", expressed either as disruptive behavior or as a deficit in organizing, structuring, and integrating. Power-related characteristics which appeared to contribute to effective functioning and supportive emotional climates are: (a) a predominance of influence based on leadership, (b) a reasonable balance of influence between mother and father, and (c) at least a moderate degree of leadership displayed by the children.

Regarding affect management in the setting, happy affect was quite common, and extremely low or extremely high levels appeared to reflect disturbance. Sadness, on the other hand, was rare and appeared to reflect extreme sensitivity. Anxiety was common and appeared to be of three types--(a) a reflection

of being high-keyed and intense but relatively in control of one's self, (b) a reflection of being high-keyed and intense but also impulsive and overly active, and (c) localized symptoms of stress. A number of the parents and a few of the children showed an excess of emotional lability. Not unexpectedly, impulsivity was more common in the children than in the parents. In most cases, impulsivity in the children was highly disruptive of the family's task performance. In the most competent families, however, family members continued to function well despite the behavior of the child. Impulsivity in parents was most often demonstrated as immature and disruptive "acting out" or showing off or as distractability in cognitive functioning.

A notable problem in some families was passivity on the part of the parent. This often appeared along with concreteness and a lack of verbal ability and communication skills.

Significance for Biomedical Research

Analyses will examine how the various competencies and dysfunctions are distributed in target and control families and how they serve to maximize or minimize the appearance of problems in the children. Such information will be useful in the planning of interventions.

Proposed Course

As this study is part of a larger longitudinal study of affectively ill parents and their offspring, the children's perceptions of family relationships and qualities of family and individual functioning during family interaction will be examined developmentally, as a function of the children's and their parents' previous psychiatric status and as a function of behavioral functioning observed during previous periods of assessment. Relations of measures from this study also will be considered in terms of concurrently assessed psychiatric and behavioral measures. Data have been coded, and ratings have been made. Data analysis is to commence.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02562-02 dLDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HPA Axis Function in Offspring of Depressed Mothers and Normal Control Mothers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: G. Chrousos Chief Pediatric Endocrinology Sec. DEB NICHD

OTHERS: G. Municchi Visiting Fellow LDP NIMH
P. Martinez Senior Staff Fellow LDP NIMH
F. Putnam Senior investigator LDP NIMH
M. Radke-Yarrow Chief LDP NIMH

COOPERATING UNITS (if any)

Developmental Endocrinology Branch, NICHD

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.65

PROFESSIONAL:

.45

OTHER:

.20

CHECK APPROPRIATE BOXES:

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The hypothalamic-pituitary-adrenal (HPA) axis of adult patients with depression shows a pattern characterized by basal hypercortisolism, associated with an attenuated plasma ACTH response to ovine corticotropin-releasing hormone (oCRH). Most likely this hypercortisolism represents a defect at or above the level of the hypothalamus resulting in hypersecretion of CRH. In this study we propose to determine whether the offspring of affectively ill mothers (unipolar and bipolar) show a similar pattern of response to CRH stimulation; we will compare their response to that of offspring of normal control mothers. A standard oCRH stimulation test will be performed by administering a bolus injection of 1 mcg/Kg body weight of ovine CRH at 8 PM. Plasma levels of ACTH and cortisol will be measured before, during and after oCRH administration.

Project Description:

The hypothalamic-pituitary-adrenal (HPA) axis of adult patients with depression shows a pattern characterized by basal hypercortisolism, associated with an attenuated plasma ACTH response to ovine corticotropin-releasing hormone (oCRH). Most likely this hypercortisolism represents a defect at or above the level of the hypothalamus resulting in hypersecretion of CRH. In this study we propose to determine whether the offspring of affectively ill mothers (unipolar and bipolar) show a similar pattern of response to CRH stimulation; we will compare their response to that of offspring of normal control mothers. A standard oCRH stimulation test will be performed by administering a bolus injection of 1 mcg/Kg body weight of ovine CRH at 8 PM. Plasma levels of ACTH and cortisol will be measured before, during and after oCRH administration.

Methods and Findings:

Sixty children, ages 9-15 years old, are enrolled in this study: twenty normal offspring of bipolar mothers (10 boys and 10 girls), twenty normal offspring of unipolar mothers (10 boys and 10 girls), and twenty normal offspring of normal control mothers (10 boys and 10 girls). Subjects represent a subsample of children currently participating in an ongoing study of the developmental disorders of offspring of parents with and without affective disorders (study number 79-M-123). These children and their parents have already undergone psychiatric assessments to determine their DSM-III-R diagnostic classification. A medical history has been previously obtained and a physical examination has been performed by a staff pediatrician to rule out major medical illnesses.

The subjects are given a physical examination and a blood sample (10 ml) is drawn for SMAC, CBC, and thyroid function tests (T_e, T_r, free T_r, TSH, TBG). Subsequently, a heparin lock is placed for multiple blood sampling at 7 PM. Ovine CRH (1mcg/Kg) is injected intravenously over 2 minutes beginning at 8PM (time 0). Blood (5 ml) is drawn at -15, 0, 30, 60, 90, 120 and 180 minutes for measurement of plasma ACTH and cortisol. Because ACTH and cortisol levels may increase following the stress of venipuncture, the heparin lock is placed at least 50 minutes prior to CRH administration. During the test, a nurse is present. A physician is available in-house for the 30 mins. following the administration of CRH.

Proposed Course:

Data collection has been completed. Analyses of data will proceed.

Significance to Biomedical Research:

We hypothesize that behaviorally normal offspring of affectively ill mothers (unipolar and bipolar) have a different pattern of hormonal response to oCRH, in comparison to normal offspring of normal control mothers. Ultimately, and if this is true, the CRH stimulation test might be used as a diagnostic tool for early detection of those individuals that might be prone to develop an affective illness later in life.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02604-01 LDP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cognitive Risks in Preschoolers at Risk for Disruptive Behavior Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	P. Cole	Senior Staff Fellow LDP NIMH
OTHERS:	F. Putnam	Medical Officer LDP NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.40	.20	.20
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The purpose of this project is the identification of <u>cognitive risks in preschool age children</u> who are at risk for developing <u>disruptive behavior disorders</u> . Decades of research on the relations between school difficulties, learning disabilities, and intelligence and disruptive behavior disorders have demonstrated modest, significant relations but precise understanding of the nature of these relations remains to be determined. Advances in cognitive and neurosciences provide new avenues of examining specific dimensions of cognitive functioning. In this project, three cognitive domains were assessed: <u>verbal, visuospatial, and executive functioning</u> . These domains test hypotheses that disruptive behavior disorders, at least for some children, are associated with difficulty with (a) verbal mediation of behavior, (b) processing of nonverbal socioemotional information, and (c) abilities in inhibiting behavioral and emotional impulses and focusing and planning appropriate actions and problem-solving.		

Project Description

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Methods and Findings

A large battery of cognitive tests were administered to a sample of preschoolers were classified as at high, moderate, or low risk for the development of disruptive behavior disorders on the basis of parent and teacher reports (from project Z01 MH 02448-04. The battery included the McCarthy Scales of Children's Abilities (a general intelligence test with subscales for verbal and nonverbal functioning), the Florida Kindergarten Screening Battery (a screening battery for early detection of risk for learning disabilities with verbal and nonverbal subtests), and a battery of experimental tasks designed to assess additional verbal, nonverbal, and executive functions. The first step in data reduction analyses is the identification of coherent relations among different tests in order to identify aggregate measures tapping each cognitive construct. Principal component analyses of the battery of experimental tasks designed to tap executive function has shown that six or seven measures produced a single component. Currently, similar analyses are underway to examine verbal and nonverbal domains. A preliminary discriminant function analysis indicates that the executive function component has a better than chance ability to discriminate children as a function of their at-risk classification. More importantly, the battery may identify a subgroup of children with difficulties in particular cognitive operations and in behavioral control, particularly in terms of overactivity rather than aggressivity.

Significance to Biomedical Research and to the Program of the Institute

Scientific information on the specific cognitive functions associated with disruptive behavior disorders, in children prior to their entry into elementary school, contributes to efforts to understand the heterogeneous influences leading to conduct disorder. For some children cognitive factors may indicate neurodevelopmental vulnerabilities. These data examine the contribution of certain cognitive risks in the development of stable disruptive behavior and associated psychiatric disorders.

Proposed Course

Time 2 data collection will continue through 1993, the year in which the second group of children are completing first grade.

Related Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02605-01 LDP																
PERIOD COVERED October 1, 1991 through September 30, 1992																		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Emotion Regulation in Preschoolers at Risk for Disruptive Behavior Disorders																		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">P. Cole</td> <td style="width: 35%;">Senior Staff Fellow</td> <td style="width: 15%;">LDP NIMH</td> </tr> <tr> <td>CO-PI:</td> <td>C. Zahn-Waxler</td> <td>Chief, Section on Child Behavior Disorders</td> <td>LDP NIMH</td> </tr> <tr> <td>OTHERS:</td> <td>F. Putnam</td> <td>Medical Officer</td> <td>LDP NIMH</td> </tr> <tr> <td></td> <td>N. Fox</td> <td>Professor</td> <td>U. of Maryland</td> </tr> </table>			PI:	P. Cole	Senior Staff Fellow	LDP NIMH	CO-PI:	C. Zahn-Waxler	Chief, Section on Child Behavior Disorders	LDP NIMH	OTHERS:	F. Putnam	Medical Officer	LDP NIMH		N. Fox	Professor	U. of Maryland
PI:	P. Cole	Senior Staff Fellow	LDP NIMH															
CO-PI:	C. Zahn-Waxler	Chief, Section on Child Behavior Disorders	LDP NIMH															
OTHERS:	F. Putnam	Medical Officer	LDP NIMH															
	N. Fox	Professor	U. of Maryland															
COOPERATING UNITS (if any) University of Maryland																		
LAB/BRANCH Laboratory of Developmental Psychology																		
SECTION Section on Child Behavior Disorders																		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892																		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:																
.55	.20	.35																
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews																		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The purpose of this project is the identification of pattern of <u>emotion regulation</u> in young children as a function of thier <u>risk</u> for developing <u>disruptive behavior disorders</u>. These disorders have been studied as behavioral disturbances and the nature of the associated affective disturbances have not been studied. This project examines the regulation of emotion, particularly anger, in relation to behavioral difficulties in preschool age children. Observational and self-report data from experimental tasks that challenge self-control, and internal state changes during exposure to specific emotion stimuli, were collected. These data address the emotional dynamics underlying the quality of preschoolers' behavior under conditions of <u>disappointment, frustration, and temptation</u>. Psycho-physiological data assess changes in <u>heart rate, vaqal tone, and skin conductance</u> as a function of exposure to specific emotion stimuli. Preliminary analyses indicate that preschoolers reveal more negative affect and more disruptiveness in frustrating situations than low risk preschoolers, particularly when an adult is present. Negative emotion appears to precede disruptiveness. </p>																		

Project Description

The purpose of this project is the identification of patterns of emotion regulation in young children as a function of their risk for developing disruptive behavior disorders. These disorders have been studied as behavioral disturbances and the nature of the associated affective disturbances have not been studied. When intrapsychic processes are examined, they usually emphasize cognitive dimensions (e.g., attributional style, problem-solving skills). Difficulties in the emotional domain are evidenced however in terms of angry, hostile behavior and in the high rate of comorbidity of conduct problems with depression and anxiety disorders. This study examines the regulation of emotion, particularly anger and sadness, in relation to behavioral difficulties in preschool age children. Observational and self-report data from experimental tasks that challenge self-control, and internal state changes during exposure to specific emotion stimuli, were collected. Observational procedures examine the emotional dynamics of adaptive and maladaptive behaviors under conditions of disappointment, frustration, and temptation. Specifically, the latency to angry affect, the intensity and duration of angry episodes, the proportion of anger to sad or anxious affect, and temporal relation of negative affect to behavioral disruptiveness are examined. Psychophysiological data assess changes in heart rate, vagal tone, and skin conductance as a function of exposure to specific emotion stimuli.

Methods and Findings

Approximately 80 preschool age children were classified as at high, moderate, or low risk for the development of disruptive behavior disorders on the basis of parent and teacher reports. These children were recruited under project Z01 MH 02448-04. Children whose behavior ratings were 2 standard deviations above the norm for their gender and age group were identified as at high risk, 1 standard deviation above the norm as at moderate risk. Children whose ratings were 1 standard deviation above the norm were identified as at moderate risk. Children who showed no indications of significant behavior problems were classified as at low risk.

Three observational procedures were used to assess emotion regulation during self-control challenges. On separate occasions, preschoolers (a) were asked by their mothers to wait to open a surprise until she finished some work, (b) given an undesirable prize after they completed some work, and (c) were told not to touch a jar full of candy and toys while the experimenter was absent. Affect and behavior coding systems were developed to assess the quality of the child's emotional displays and behavior during each challenge. Piloting of the affect coding system, which is based on nonverbal emotionally expressive behavior, was conducted with a sample of two-year-olds in emotionally challenging situations. On another occasion, psychophysiological data (heart period, vagal tone, and skin conductance) were assessed during a baseline period and during a videotape of a peer experiencing happy, sad, angry, and scared emotions. In order to provide experimentally controlled mood inductions, a videotape was developed at LDP.

Preliminary analyses of the observational data from the waiting, disappointment and temptation procedures indicate that preschoolers who are at moderate and high risk for the development of disruptive behavior disorders are significantly more likely to display negative emotions, particularly anger, less likely to display the positive emotion of joy, and more likely to engage in disruptive during the challenging situations than their low risk peers. Regarding the dynamics of these emotional reactions, three findings have emerged to date. First, risk group differences exist only in the presence of an adult. Specifically, these children are less likely to display joy, more likely to display anger and more likely to engage in disruptive behaviors (aggression, limit-testing) when required to wait for prize in the presence of their mothers and receiving an undesired prize from a staff member. Left alone with the undesired prize, all children display equivalent amounts of frustration and disappointment. At-risk preschoolers do touch forbidden objects at a significantly higher rate than low risk preschoolers.

Second, negative emotional displays precede the onset of disruptive behavior as opposed to accompanying or following them. These data suggest that emotionality may play a facilitative role in the display of aggressive, noncompliant behavior. Third, the children do not differ generally in terms of the intensity of their emotional displays. Analyses of latency and specific emotion intensities have not been completed. In general, these data suggest that there are specific difficulties in the area of emotional and behavioral regulation that are associated with preschool age behavioral difficulty and that these are particularly salient in interpersonal contexts.

Two sets of preliminary analyses have been conducted with the psychophysiological data. First, the newly developed mood induction procedure was tested to see whether effects of story emotion could be seen in the general sample. These analyses indicate differential changes in heart rate, vagal tone, and skin conductance as a function of emotion in the expected directions. Second, baseline psychophysiological data taken prior to the mood induction procedure were evaluated to determine whether there are any risk group differences in resting baseline. The data suggest no such differences between the groups. Analyses examining individual differences in reactivity to these stimuli are currently underway.

Significance to Biomedical Research and to the Program of the Institute

Scientific information on the role of emotional dysfunction in the etiology of conduct disorders, particularly in terms of early childhood development, provides new information that can inform us about the precise nature of how risk develops in children and can suggest avenues of early intervention and prevention of disruptive behavior disorders. This sample is part of a longitudinal project, permitting the evaluation of these patterns of emotion regulation as predictors of stable and transient behavior problems and the development of symptoms associated with actual diagnoses.

Proposed Course

Time 2 data collection will continue through 1993, the year in which the second group of children are completing first grade. To examine the continuity of emotional styles of coping, similar data are being collected during the first grade evaluation. After identifying patterns of emotion regulation in the sample, it will be useful to study the relation between these patterns and measures of child temperament, of family functioning (e.g., exposure to marital discord, stressful life events), and parenting practices. Upon completion of the Time 2 data collection, the emotion regulation data will be examined in an effort to construct aggregate variables that serve to describe the child's disposition and to be used in the predictive model of the development of sustained behavioral problems.

Related publications

Cole, P.M., & Zahn-Waxler, C. (in press). Emotional dysregulation in disruptive behavior disorders. In D. Cicchetti & S. Toth (Eds.), Rochester Symposium on Developmental Psychopathology, Vol 4: A developmental approach to affective disorders. Rochester: University of Rochester Press.

Cole, P.M., Barrett, K.C., & Zahn-Waxler, C. (1992). Emotion displays in toddlers during mishaps. Child Development, 63, 314-324.

Cole, P.M., Woolger, C., Power, T.G., & Smith, K.D. (in press). Parenting difficulties among adult survivors of father-daughter incest. Child Abuse and Neglect.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02606-01 LDP
PERIOD COVERED October 1, 1991 through September 30, 1992		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Sibling Relationships in Families with Affectively Ill and Well Parents		
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)		
PI:	L. Tarullo	Staff Fellow LDP NIMH
OTHERS:	M. Radke-Yarrow	Chief LDP NIMH
COOPERATING UNITS (if any) NONE		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892		
TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
.40	.20	.20
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) In the longitudinal study of affectively ill and well parents, parent-child relationships have been evaluated in early childhood (e.g., MH 02487, MH 02409), late childhood and early adolescence (MH 02488). The relationship of the two siblings in the family is also of interest in assessing the socioemotional development of children at risk for affective illness. Observations of sibling interaction were made at the beginning of the study, when the younger sibling was between 1 1/2 and 3 1/2 years of age and the older sibling was between 5 and 8 years of age, and again at two later developmental periods. The objective of the study is to examine patterns of interaction in the areas of affect, communication, and play behavior in these sibling pairs across the three developmental periods in terms of parental psychopathology, child problems, and gender composition of the dyad. In addition, children's representations of the sibling relationship will be considered.		

Project Description:

In the longitudinal study of affectively ill and well parents, parent-child relationships have been evaluated in early childhood (e.g., MH 02487, MH 02409), late childhood and early adolescence (MH 02488). The relationship of the two siblings in the family is also of interest in assessing the socioemotional development of children at risk for affective illness. Observations of sibling interaction were made at the beginning of the study, when the younger sibling was between 1 1/2 and 3 1/2 years of age and the older sibling was between 5 and 8 years of age, and again at two later developmental periods. The objective of the study is to examine patterns of interaction in the areas of affect, communication, and play behavior in these sibling pairs across the three developmental periods in terms of parental psychopathology, child problems, and gender composition of the dyad. In addition, children's representations of the sibling relationship will be considered.

Methods:

One hundred sibling pairs, participants in the longitudinal study of families with well and affectively ill parents, are observed at three developmental periods. At the first time of measurement, the younger sibling is 1 1/2 to 3 1/2 years old and the older sibling is 5 to 8 years old. At the second time period, the younger is 5 to 6 years old and the older is 8 to 11 years old. At the third time period, the younger is 8 to 11 years old and the older is 12 to 16 years old. Sibling pairs represent four gender configurations: same-sex male, same-sex female, older female and younger male, and older male and younger female. During each data collection period, the siblings are left alone for 20 minutes in a comfortable research apartment with age-appropriate toys available and asked to play together while the parent or parents talk with experimenters. The resulting videotaped interactions will be coded for nature of play (independent, parallel, joint) and level of play (manipulative, symbolic), as well as for quality of interaction on such dimensions as affect, communication, caregiving, competition, conflict, and cooperation. A related measure taken at the third developmental period is a representation of each child's satisfaction with sibling emotional support, instrumental help and companionship, as well as the child's report on amount of sibling conflict. This combination of behavioral and self-report measures from the two children will provide multiple perspectives on the relationship.

Significance to Biomedical Research:

In families at risk, the sibling relationship may provide compensatory support or additional relational stress. Understanding the nature of sibling interaction provides an additional measure of family dynamics in affectively ill families.

Proposed Course:

A coding system has been developed to assess affect, communication, and play behavior for the sibling dyads across three developmental periods. Coding will begin during the summer with the assistance of summer research interns. Data analysis will take into account parental psychopathology, child problem status, and gender composition, as well as each child's representation of the relationship.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02607-01 LDP			
PERIOD COVERED October 1, 1991 through September 30, 1992					
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Socialization Experiences of Young Children with Conduct Problems					
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 33%; vertical-align: top;"> PI: C. Zahn-Waxler OTHERS: P. Cole S. Denham C. Weissbrod </td> <td style="width: 33%; vertical-align: top;"> Chief, Section on Child Behavior Disorders Senior Staff Fellow Professor Professor </td> <td style="width: 33%; vertical-align: top;"> LDP NIMH LDP NIMH George Mason University The American University </td> </tr> </table>			PI: C. Zahn-Waxler OTHERS: P. Cole S. Denham C. Weissbrod	Chief, Section on Child Behavior Disorders Senior Staff Fellow Professor Professor	LDP NIMH LDP NIMH George Mason University The American University
PI: C. Zahn-Waxler OTHERS: P. Cole S. Denham C. Weissbrod	Chief, Section on Child Behavior Disorders Senior Staff Fellow Professor Professor	LDP NIMH LDP NIMH George Mason University The American University			
COOPERATING UNITS (if any) George Mason University The American University					
LAB/BRANCH Laboratory of Developmental Psychology					
SECTION					
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892					
TOTAL STAFF YEARS: .70	PROFESSIONAL: .20	OTHER: .50			
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews					
SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.) <p> <u>Family dynamics, child-rearing practices, and parent-child interaction patterns</u> are assessed in families with 4-5 year old children who vary in degree of <u>risk for conduct problems</u>. Research has focussed principally on negative socialization practices that establish and maintain misconduct. The current work emphasizes, in addition, <u>proactive parenting practices</u> that may minimize the need for reactive, coercive control. Proactive practices refer to anticipatory guidance; positive, educative exchanges; and structuring and organizing the environment so as to facilitate the development of critical social skills as well as to encourage appropriate compliance. The parent's affective style with the child is also examined, i.e. the specific emotions shown and the emotional sensitivity to the child's developmental level. Parent-child interaction is assessed under naturalistic conditions and in a series of dyadic and triadic tasks that stimulate and challenge the family. Two coding systems have been developed to evaluate proactive parenting and affective styles and their reliability is being assessed. A third system that focuses on family dynamics and processes will also be developed. A basic goal is to identify socialization approaches that predict moderation and <u>remission of problem behaviors over time</u>, in order to inform strategies for <u>early intervention</u>. </p>					

Project Description

Family dynamics, child-rearing practices and parent-child interaction patterns are assessed in families with 4-5 year old children who vary in degree of risk for conduct problems. Research has focussed principally on negative socialization practices that establish and maintain misconduct. The current work emphasizes, in addition, proactive parenting practices that may minimize the need for reactive, coercive control, e.g. anticipatory guidance; positive, educative exchanges; structuring and organizing the environment so as to facilitate the development of critical social skills and to encourage appropriate compliance. The purpose of this approach is to identify socialization patterns that predict remission and moderation of problem behaviors over time.

Methods Employed and Major Findings

Over 80, 4-5 year-old children who vary in risk for conduct problems are observed with their parents in a laboratory setting. Children at risk are oppositional, defiant, and difficult to manage. All children are part of a larger, longitudinal investigation that focuses on prediction of problem behaviors over time (Z01 MH 02448-03 LDP). This study provides detailed assessments of the child-rearing environments experienced by children who differ in their risk status. In one observation session, parent-child dyads and triads are evaluated during a series of challenging, stimulating tasks. They are also observed together under more naturalistic conditions (e.g. having a snack together, reading, playing). Negative and proactive socialization patterns are scored as well as the parents' emotional styles. Reliability of these coding systems is currently being assessed. A third scoring system for assessing family dynamics and processes (e.g. alliances, triangulation) is under development. Parental attitudes, values and practices are scored using the Block Q Sort procedure. Other features of family climate are examined with self-report and questionnaire methods. This includes parental depression, personality characteristics, marital conflict, and familial and extra-familial stress. Of special interest is how these 'environmental' influences (from the child's perspective) interact with specific observed parental rearing practices, to predict given child outcomes over time.

Proposed Course

Children are currently being evaluated for behavior problems and psychiatric status after they have made a transition into first grade. Child-rearing practices are examined again at this time. Because children and parents are seen over time, it will be possible to better determine the particular combinations of global and specific characteristics of parenting that contribute to continuity and change in problem behaviors.

Significance to Biomedical Research and the Institute Program

Conduct problems, often begin early in life and once in place, are very resistant to change. Interventions at this time would help to prevent negative, coercive parent-child interactions from escalating to produce entrenched problem behaviors in children. Effective child behavior management requires skilled parenting patterns. Basic research on parenting behaviors and characteristics of caregivers who succeed over time with young children who are difficult-to-manage, helps to identify those practices that are most effective. Hence, they inform intervention strategies.

Publications

Zahn-Waxler, C, Denham, SA, Iannotti, RJ, Cummings, EM. Peer relations in children with a depressed

caregiver. In RD Parke and GW Ladd (Eds), Family-Peer Relationships: Modes of Linkage, Hillsdale, NJ: Erlbaum Press, in press.

Zahn-Waxler, C. and Wagner, E. (in press). Caregivers' interpretations of infant emotions: A comparison of depressed and well mothers. In R Emde, J Osofsky, and P Butterfield (Eds), Parental perception of infant emotions. Washington, DC: Clinical Infant Report Series.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02608-01 LDP								
PERIOD COVERED October 1, 1991 through September 30, 1992										
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Adaptive Development in the Offspring of High Risk Families										
PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) <table style="width: 100%; border: none;"> <tr> <td style="width: 20%;">PI:</td> <td style="width: 40%;">M. Radke-Yarrow</td> <td style="width: 20%;">Chief</td> <td style="width: 20%;">LDP NIMH</td> </tr> <tr> <td>CO-PI:</td> <td>E. Brown</td> <td>Staff Fellow</td> <td>LDP NIMH</td> </tr> </table>			PI:	M. Radke-Yarrow	Chief	LDP NIMH	CO-PI:	E. Brown	Staff Fellow	LDP NIMH
PI:	M. Radke-Yarrow	Chief	LDP NIMH							
CO-PI:	E. Brown	Staff Fellow	LDP NIMH							
COOPERATING UNITS (if any)										
LAB/BRANCH Laboratory of Developmental Psychology										
SECTION										
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland 20892										
TOTAL STAFF YEARS: 1.30	PROFESSIONAL: .80	OTHER: .50								
CHECK APPROPRIATE BOXES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews										
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) This is a study of "resilient" children of affectively ill parents. They are children who, despite high risk conditions of both genetic and environmental origins show high levels of adaptive behavior. The objectives are to (a) identify variables in child characteristics and interpersonal relationships that distinguish these children from children with similar risks who develop serious problems; and (b) determine the stability of their adaptations, as well as the turning points in their developmental trajectories. Children were identified from the longitudinal sample who are especially at high risk: having two affectively ill parents, a family history of affective illness, family conditions characterized by chaos and multiple stress. The children who were functioning well (based on multiple criteria) in late childhood or adolescence are being investigated through standard case studies, using the battery of measures obtained prospectively, beginning in early childhood. Their histories are compared with the histories of children of similar high risk who have developed serious problems.										

Project Description

This is a study of "resilient" children of affectively ill parents. They are children who, despite high risk conditions of both genetic and environmental origins show high levels of adaptive behavior. The objectives are to (a) identify variables in child characteristics and interpersonal relationships that distinguish these children from children with similar risks who develop serious problems; and (b) determine the stability of their adaptations, as well as the turning points in their developmental trajectories.

Methods and Findings:

Children were identified from the longitudinal sample who are especially at high risk: having two affectively ill parents, a family history of affective illness, family conditions characterized by chaos and multiple stress. The children who were functioning well (based on multiple criteria) in late childhood or adolescence are being investigated through standard case studies, using the battery of measures obtained prospectively, beginning in early childhood. Their histories are compared with the histories of children of similar high risk who have developed serious problems. From 130 offspring of affectively ill mothers, 18 met the criteria for "resilience" under extreme conditions of risk.

Significance to Biomedical Research:

Not all children follow the course of development that the theories predict. Understanding the cases that diverge can lead to better understanding of pathogenic and healthy developmental processes, and suggest strategies for intervention.

Proposed Course:

Analyses of the standard case data sets on each child are underway. Interviews with the children are planned to obtain (a) the children's understanding or view of their parents' illness, and (b) their self-assessment of how they have coped with it.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 02609-01 LDP

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

HPA Axis Function in Conduct Disorder Children.

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	G. Municchi	Visiting fellow	LDP NIMH
OTHERS:	P. Cole	Senior Staff Fellow	LDP NIMH
	C. Zahn-Waxler	Chief, Section on Child Behavior Disorders	LDP NIMH
	S. Suomi	Chief	LCE NICHD
	G. Brown	Clinical Director	NIAAA
	M. Linnoila	Scientific Director	NIAAA

COOPERATING UNITS (if any)

Laboratory of Comparative Ethology, NICHD
Laboratory of Clinical Studies, NIAAA

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

.50

PROFESSIONAL:

.20

OTHER:

.30

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Several studies have shown that a large proportion of patients with major depressive disorders exhibit hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis function, expressed by elevated plasma cortisol concentrations. We hypothesize that behavior problem children, particularly those who show concurrent symptoms of depression and anxiety, may have hyperactivity of HPA axis function, as indicated by elevated plasma and salivary cortisol levels. Approximately 80 conduct problem children, aged 6-7 years, will undergo a physical examination in order to get extensive pediatric history (perinatal, developmental and general medical history); in addition, plasma ACTH, plasma and salivary cortisol levels will be assessed. This provides the opportunity to evaluate the HPA axis function in these children, to evaluate the correlation between plasma and salivary cortisol levels and to assess cortisol response under stress and nonstress conditions. Such information would be useful in advancing the early screening of children at risk for disruptive behavior disorders and affective disorders.

Project description

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Methods and findings

Approximately 80 behavior problem children, aged 6-7 years, will undergo a physical examination to get extensive pediatric history (perinatal, developmental and general medical history) and to assess growth, blood pressure and routine blood chemistries. A blood sample will be collected for screening chemistries and for assessment of plasma ACTH and cortisol levels. Three saliva samples, to assess salivary cortisol levels, will also be collected: one on a day prior to the physical examination (collected at home between 8 and 9 AM), one on the morning of the physical examination (collected in the lab between 8 and 9 AM), and one immediately following the physical examination and venipuncture (around 10 AM). This will permit assessment of the correlation between plasma and salivary cortisol, and assessment of the cortisol response under stress and nonstress conditions. Included are self-reports of pain experienced during the procedure of venipuncture, and a behavior rating using an observational scale indicative of distress and anxiety.

Proposed course

Data collection is ongoing. To date, approximately 40 children have been assessed.

Significance to Biomedical Research and the Program of the Institute

Evaluation of hypothalamic-pituitary-adrenal (HPA) axis function, through assessment of plasma ACTH and cortisol levels and salivary cortisol levels, in behavior problem children, particularly those who show concurrent symptoms of depression and anxiety, would be useful in advancing the early screening of children at risk for disruptive behavior and affective disorders.



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